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

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SUMMARY

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

Most of the anther opening processes take place in the epidermis and the endothecium. The starch in these two layers is used for different steps of the dehiscence process. The (single) middle layer is isolated from the endothecium and finally degenerates. This process is related to tapetal functions.

Sporopollenin-containing structures arise on both the tapetum and the microspores, for pollen dispersal and protection, respectively. In the tapetum, starch turns into part of the pollenkitt. During the microspore mitosis the starch of the microspores is deposited in the vegetative cell. The polarity in the microspores is related to the formation and development of the generative cell. Ultrastructural changes in the different cell layers are discussed in relation to their development and interaction.

Key-words: anther, anther dehiscence, endothecium, pollen, pollen dispersal, tapetum, tissue, interaction.

INTRODUCTION

Despite the many reports of anther development that have appeared during the last decennia, few investigations were carried out on the locule tissues other than the tapetum and the developing pollen (Keijzer & Willemse 1988), and studies of the entire developing locule as a functional unit are scarce (Reznickova & Willemse 1980).

In this report we investigate the locule development of *Gasteria verrucosa* (Mill.) H.Duval on the ultrastructural level from the free microspore stage. This work was preceded by a study of the locule development of the same species from the pollen mother cell until the free microspore stage (Keijzer & Willemse 1988) as well as other studies of detailed aspects of the anther development in *G. verrucosa* (Willemse 1972; Schröder 1985; Van Lammeren *et al.* 1985; Keijzer 1983, 1987a,b; Keijzer *et al.* 1987a,b).

MATERIALS AND METHODS

Anthers of *Gasteria verrucosa* (Mill.) H.Duval in different stages of development were fixed, observed using electron microscopy and the results were reported as described by Keijzer & Willemse (1988).

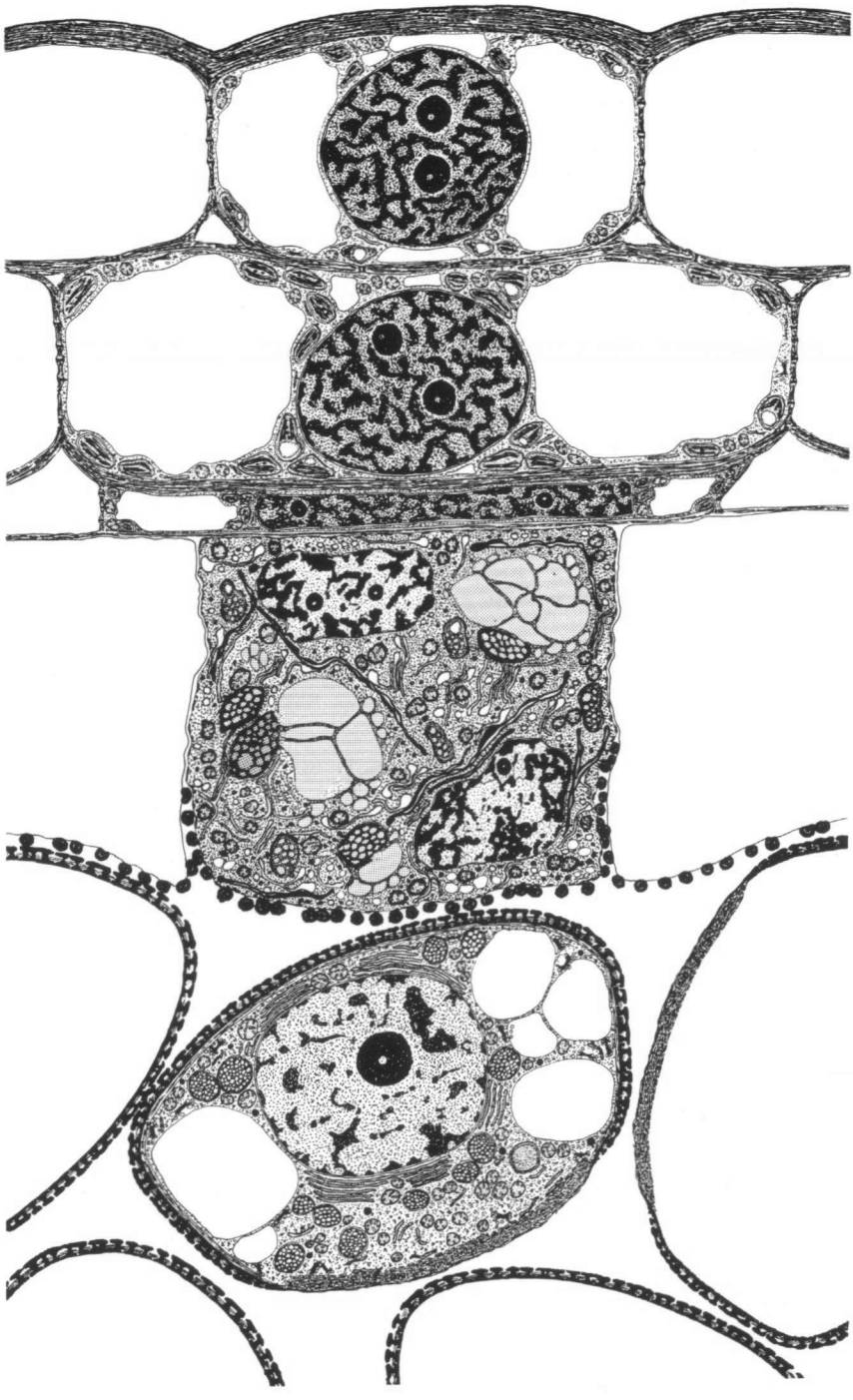


Fig. 1. The vacuolated microspore stage. The middle layer is isolated from the endothecium, their walls dissociate and the plasmodesmata disppear. The tapetum synthesizes large amounts of pollenkitt. The microspore vacuolates and the intine is deposited.

RESULTS

The vacuolated microspore stage (Fig. 1)

At this stage the plastids of the epidermis and the endothecium contain thylakoids, starch and plastoglobules, the latter colouring the anther yellowish-green (Fig. 2a). Holes are present in the wall between the endothecium and the flattened middle layer; plasmodesmata are absent (Fig. 2b).

The tapetal cells are slightly vacuolated. Their plastics contain very little starch and are mainly filled with grey material, which gradually turns into droplets and fuses into larger units (Fig. 2c). Some plastids are strongly swollen and fuse, and this is followed by the disappearance of the plastid membrane (Fig. 2d). This developmental variation can be found among different plastids of each cell, in which a developmental range can be observed. Long strands of electron dense material are bordered by swollen RER cisterns in the cytoplasm (Fig. 2e). Between the membranes of both the mitochondria and some of the plastids, electron-dense droplets can be found; dictyosomes are absent (Fig. 2c). The nuclei are relatively small, the euchromatin is electron dense, in contrast with the heterochromatin. The plasma membrane has disappeared and the cytoplasm is bordered by the tapetal membranes (Fig. 2f).

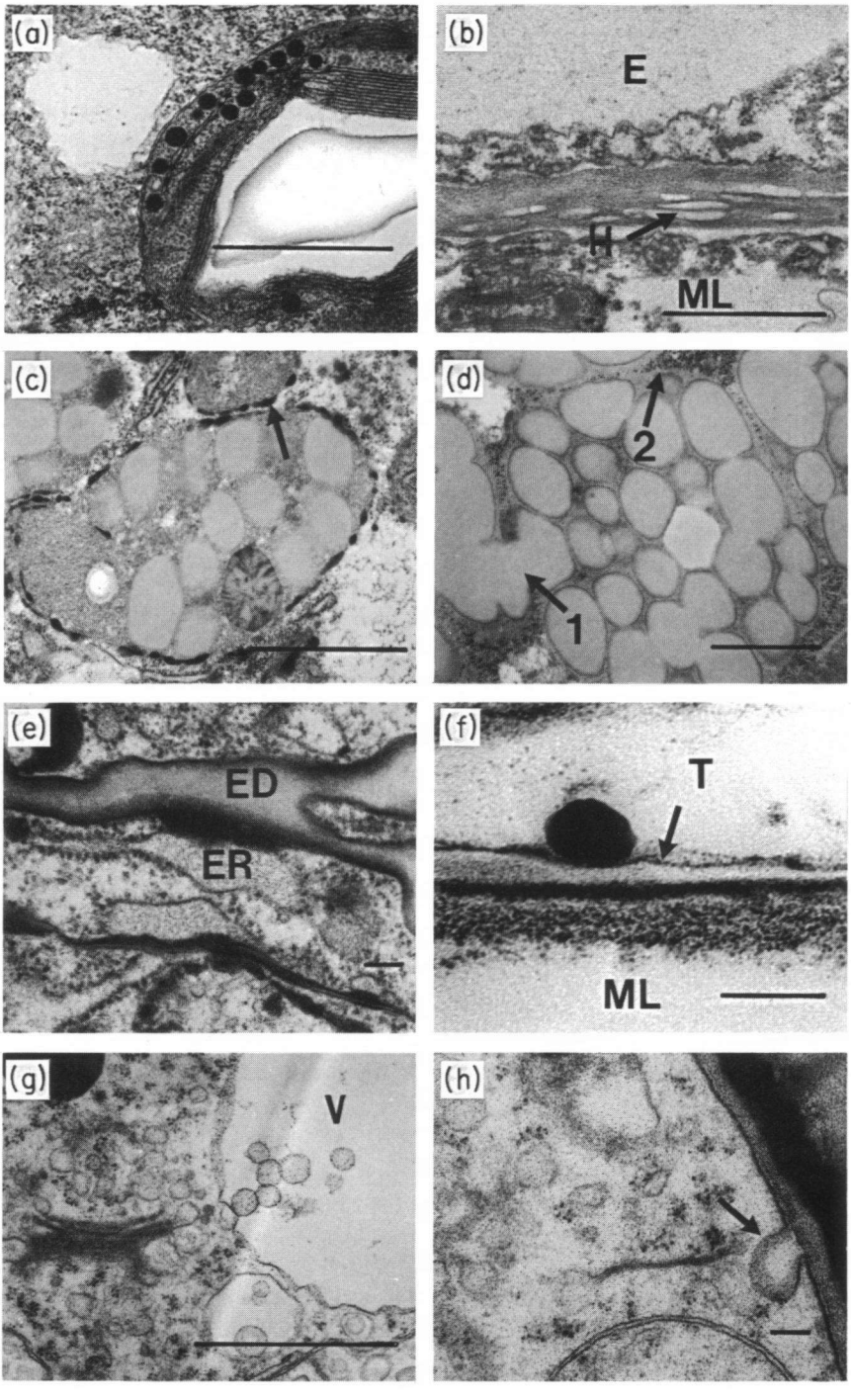
In the microspores the vacuoles fuse with, and sometimes contain, dictyosome vesicles (Fig. 2g). The intine is deposited in association with dictyosome vesicles and fuses with the plasma membrane (Fig. 2h). The thickened intine of the colpus is intruded by radially directed plasma membrane extensions, which are sometimes bordered by RER cisterns and may become isolated from the plasma membrane (Fig. 3a). Rough endoplasmic reticulum stacks border the nucleus, and the plastids contain starch (Fig. 3b). Most of the cytoplasm is found between the colpus and the nucleus, which borders the opposite wall. The vacuoles fill the other zones of the cell.

The generative cell against the intine (Fig. 4)

The locular circumference can be seen to have grown and the middle layer has flattened. In the epidermis and endothecium plastids the starch has decreased in contrast with the plastoglobules (Fig. 3c). In the epidermis cells the number of dictyosomes has increased. In the endothecium cells the nucleus and most of the plastids border the inner tangential wall (Fig. 3d). In the middle layer the nuclear chromatin becomes more electron dense and the plasma membrane undulates slightly (Fig. 3d).

The tapetal cells have shrunk and thus the concentration of ribosomes has increased. The nuclei have become smaller. The electron-dense strands fuse with the grey droplets to form large drops of pollenkitt (Fig. 3e). The numbers of both mitochrondria and RER have decreased.

A mitotic division can be observed which divides the pollen grain into a vegetative and a much smaller generative cell. The latter is mainly filled with two terminal vacuoles and a nucleus with electron dense euchromatin (Fig. 3f). The small amount of cytoplasm contains mitochondria, dicytosomes, single stranded RER, lipid droplets and ribosomes (Fig. 3f). The content of the vegetative cell is comparable with the previous stage, with the exception of the organelles that have been deposited in the generative cell. The amount of stacked RER is less than in the previous stage and can partially be found near the connection between the separation wall and the intine (Fig. 3f).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The generative cell being freed from the intine (Fig. 5)

The epidermis and endothecium cells are expanded, mainly in a radial direction, and contain larger vacuoles than in the previous stage. In the plastids of both layers the number of thylakoids and globules has increased (Fig. 3g). The cuticular surface has enlarged by the formation of electron-opaque wall ridges (Fig. 3g). In the endothecium cells, U-shaped wall thickenings are present, lined with RER (Fig. 3h). Large holes can be observed in the wall between the endothecium and the middle layer (Figs 3h and i). The plasma membrane undulates along both this wall and the wall thickenings (Fig. 3h). In the middle layer no plastids, mitochondria and vacuoles can be observed, the nucleus has degenerated, and the amounts of RER and lipid droplets have increased (Fig. 3i).

In the tapetal cells the turnover from plastids and electron-dense strands into pollenkitt has continued (Fig. 6a). The mitochondria have disappeared and the nucleus has degenerated (Fig. 6a). In the remaining cytoplasm the concentrations of RER and ribosomes are rather high, their total amounts have decreased (Fig. 6a).

The pollen grains are strongly swollen, the amount of cytoplasm has increased and the vacuoles have disappeared. The colpus is stretched and some of the plasma membrane vesicles are in open contact with the locular cavity (Fig. 6b). The generative cell has partly been pinched off from the intine since the contact sites of the separation wall and the intine have approached each other (Fig. 6c). The separation wall itself has disappeared and the two cells are only separated by their plasma membranes (Fig. 6c). The cytoplasmic content of the generative cell has remained unchanged, the cell is surrounded by a large amount of lipid droplets in the vegetative cell (Fig. 6d). In the latter, the concentration and amount of all types of organelles has strongly increased. However, the plastids are much smaller than in the previous stage and contain a few small starch grains and electron-dense material (Fig. 6d). All the RER is single-stranded with swollen ends. The vegetative nucleus is strongly heterochromatic (Fig. 6d).

The mature pollen grain (Fig. 7)

The epidermis and endothecium cells are strongly swollen. In the plastids of both layers the numbers of starch and thylakoids has decreased, in contrast with the plastoglobules (Fig. 6e). The cuticular ridges have become higher. Between the epidermis and the endothecium cells the plasmodesmata have disappeared. The inner tangential endothecium wall has strongly disintegrated in contrast with the wall thickenings; the amount of bordering RER has slightly decreased (Fig. 6f). Both the middle layer walls and most of the cell content, as well as the tapetal content, have disappeared; the tapetal membranes

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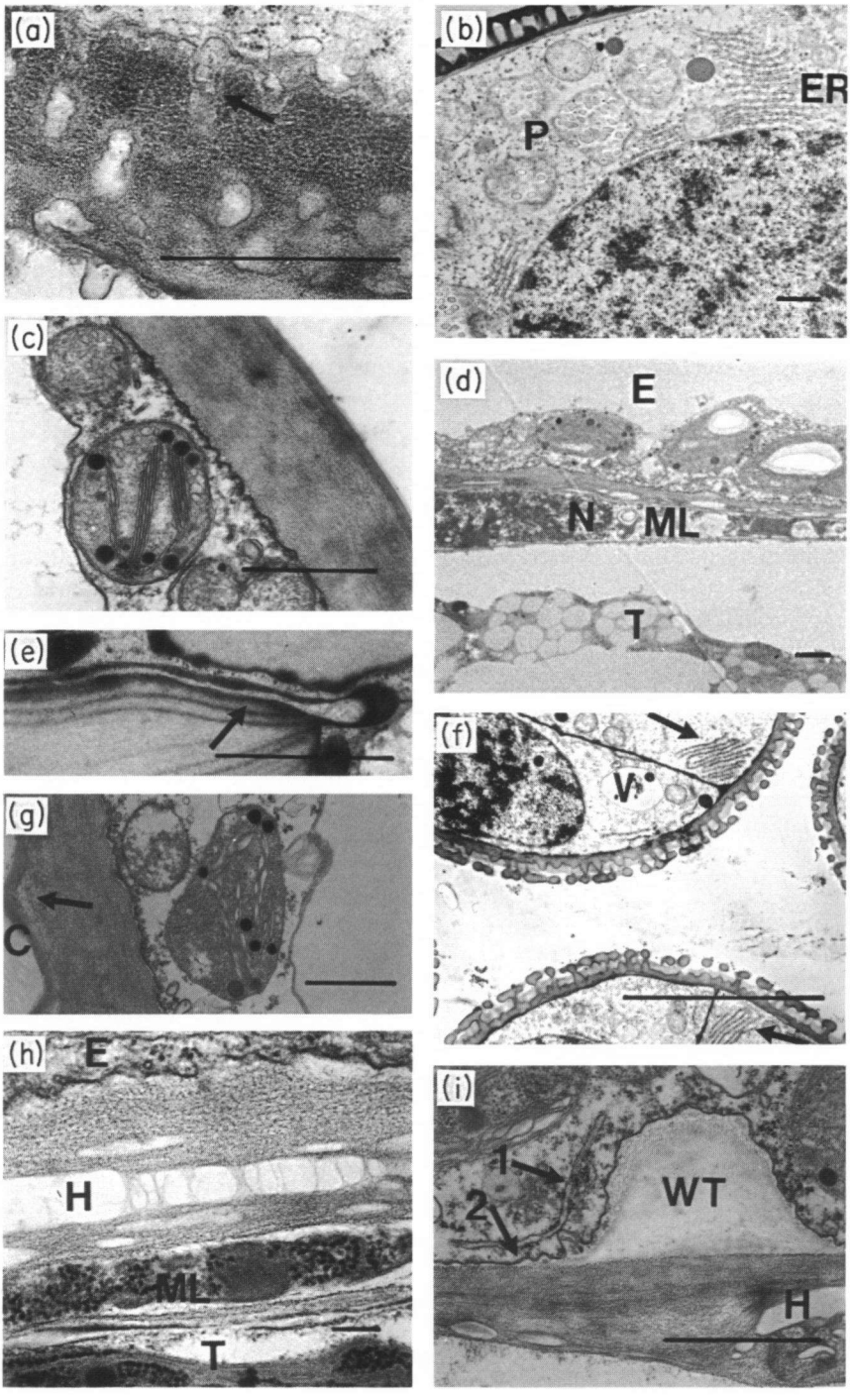
Fig. 2. (a) In the vacuolated microspore stage the plastids of the epidermis cells contain starch, thylakoids and globules, like the plastids of the endothecium (bar = 1 μm). (b) In the vacuolated microspore stage many holes (H) can be observed in the tangential wall between the endothecium (E) and the middle layer (ML) (bar = 1 μm). (c) In the vacuolated microspore stage the tapetal plastids contain droplets of grey material and a small amount of starch. Electron-dense droplets can be found (arrow) between the membranes of the plastids and the mitochondria (bar = 1 μm). (d) In the same cells the droplets shown in Fig. 3 fuse. They also fuse with those in other plastids (arrow 1). In the latter cases the plastid membranes have disappeared (bar = 1 μm). (e) In the same cells as shown in Figs 3 and 4, long electron-dense strands are bordered by swollen RER cisterns (bar = 0·1 μm). (f) In the same cells as those shown in Figs 3–5, the plasma membranes have disappeared and granular membranes (arrow), associated with electron-dense droplets, surround the cytoplasm. ML = middle layer (bar = 0·1 μm). (g) In the (vacuolated) microspores dictyosome vesicles fuse with and sometimes penetrate the vacuoles (bar = 10 μm). (h) In the same cells as those shown in Fig. 7, dictyosome vesicles fuse with the intine (bar = 0·1 μm).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border the endothecium cells (Fig. 6g). Most of the pollenkitt and some cytoplasmic remnants of both the tapetum and the middle layer (tryphine) can be found between the pollen grains and inside the exine cavities (Fig. 6h). These substances can also be found in many of the intercellular spaces between the epidermis and the endothecium cells.

The pollen grain has reached its largest size. The spindle-shaped generative cell floats freely in the vegetative cell, surrounded by stacks of RER and the lobed heterochromatic vegetative nucleus (Fig. 6i). The generative nucleus is spindle-shaped, most of the cytoplasm is found in the terminal parts of the cell. In the vegetative cell the numbers of the different organelles have increased. The mitochondria have swollen cristae (Fig. 6j). The small plastids contain electron-dense material and are lined with semi-rough ER, the smooth sides face the plastids (Fig. 6j).

DISCUSSION

The development of the epidermis and the endothecium

The gradual disappearance of the starch from the epidermis and the endothecium from stage 1 precedes structural changes in both layers. The increase in the osmotic value, which causes the strong vacuolation of the cells from stage 3 (Woycicki 1924; Keijzer 1987a), is presumably due to the breakdown of starch into smaller molecules (Woycicki 1924). Secondly, breakdown products of the starch may be used for the deposition of the wall thickenings in the endothecium (Panchanksharappa & Syamasendar 1974), which is also indicated by the migration of most of the plastids to the inner tangential wall, where the majority of the thickenings are found. Thirdly, components of the starch may be used for the formation of the carotenoid-containing plastoglobules, which colour the anther yellow. Hannig (1910) demonstrated that even weak light could raise the temperature in pigmented anthers, increasing the evaporation necessary for dehiscence (Keijzer 1983, 1987a). Furthermore, Reznickova (1983) shows a carbohydrate pathway from the epidermis and the endothecium to the tapetum and the pollen grains.

Fig. 3. (a) In the colpus area of the vacuolated microspores plasma membrane extensions (arrow) invade the thickened intine. In the cytoplasm RER cisterns border these invaginations. Some of the invaginations have been isolated from the plasma membrane (bar = 1 μ). (b) In the vacuolated microspores RER stacks (ER) border the nucleus. The plastids (P) contain many small starch grains (bar = 1 μm). (c) After the microspore mitosis the amount of starch has descreased in the plastids of the epidermis and the endothecium cells. The amount of plastoglobules has increased (bar = 1 μm). (d) After the microspore mitosis most of the organelles of the endothecium cells (E) border the inner tangential wall. The chromatin in the nucleus (N) of the middle layer cells (ML) is more electron dense than in the previous stage (bar = 1 μm). (e) After microspore mitosis, large drops of pollenkitt are present in the tapetal cells. On their borders (arrow) they fuse with the electron-dense strands shown in Fig. 2e, which sometimes contain ribosomes (bar = 1 μm). (f) After microspore mitosis the generative cell is mainly filled with a nucleus and vacuoles (V). Stacks of RER (arrows) can be found in the vegetative cell near the connection between the division wall and the intine (bar = 10 μm). (g) In the stage in which the generative cell is pinched off from the intine, the amounts of thyllakoids and plastoglobules in the epidermis (and in the endothecium) are larger than in the previous stage. The cuticle (C) undulates due to electron transparent wall ridges (arrow) (bar = 1 μm). (h) Cross-section through the part of a U-shaped wall thickening (WT) of the endothecium cells that borders the inner tangential wall. The latter contains large holes (H). The wall thickening is bordered by an RER cistern (arrow 1). The plasma membrane of the endothecium cell undulates (arrow 2). The same stage as (g) (bar = 1 μm). (i) The same holes (H) as in (h). The cytoplasm of the middle layer (ML) has degenerated, and is mainly filled with ribosomes and lipid droplets. T = tapetum. The same stage as in (g) and (h) (bar = 0·1 μm).

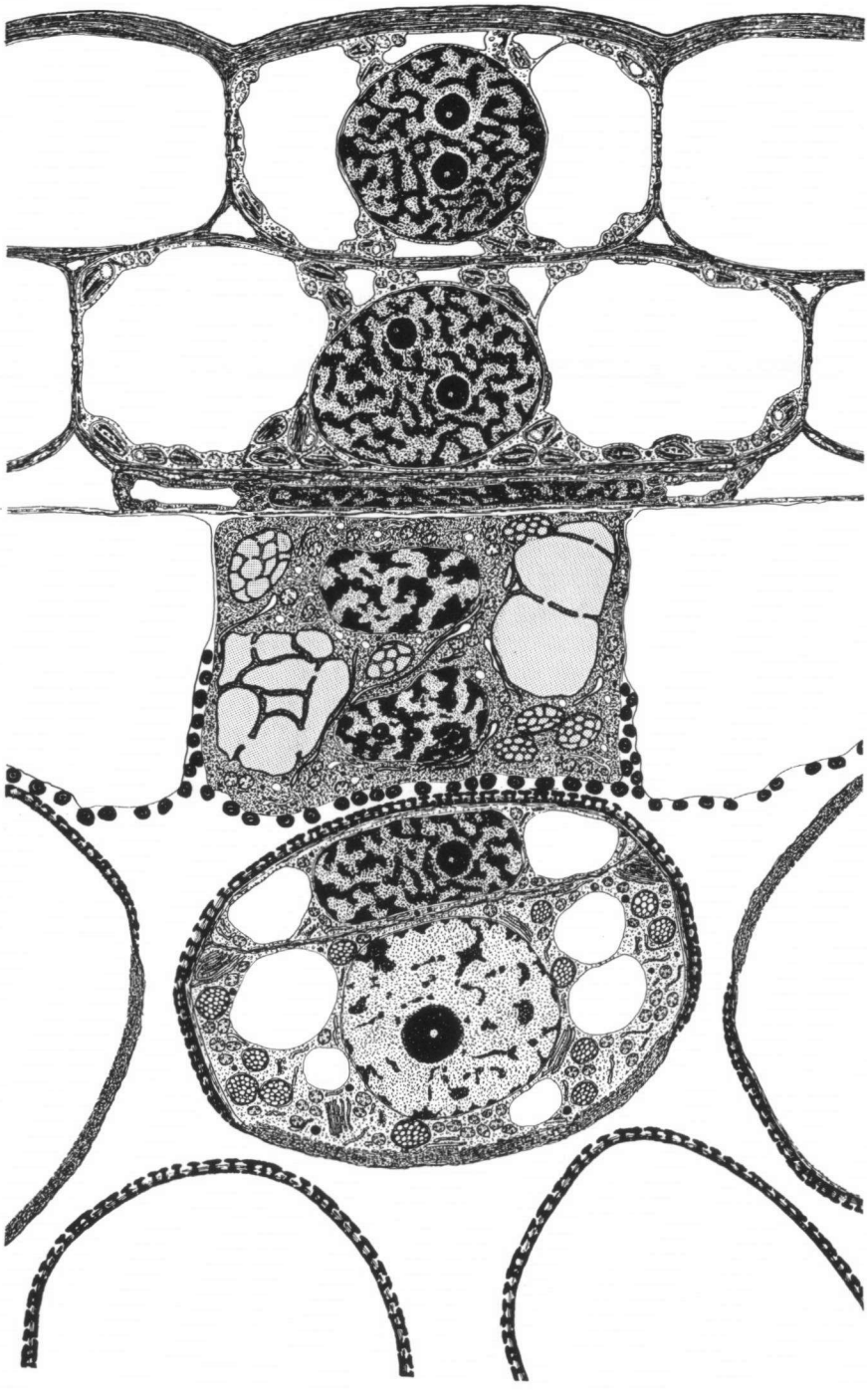


Fig. 4. The generative cell against the intine. Tapetal degeneration continues. A callosic wall separates the generative from the vegetative cell; this is probably a sign of divergent development. The former cell received no plastids.

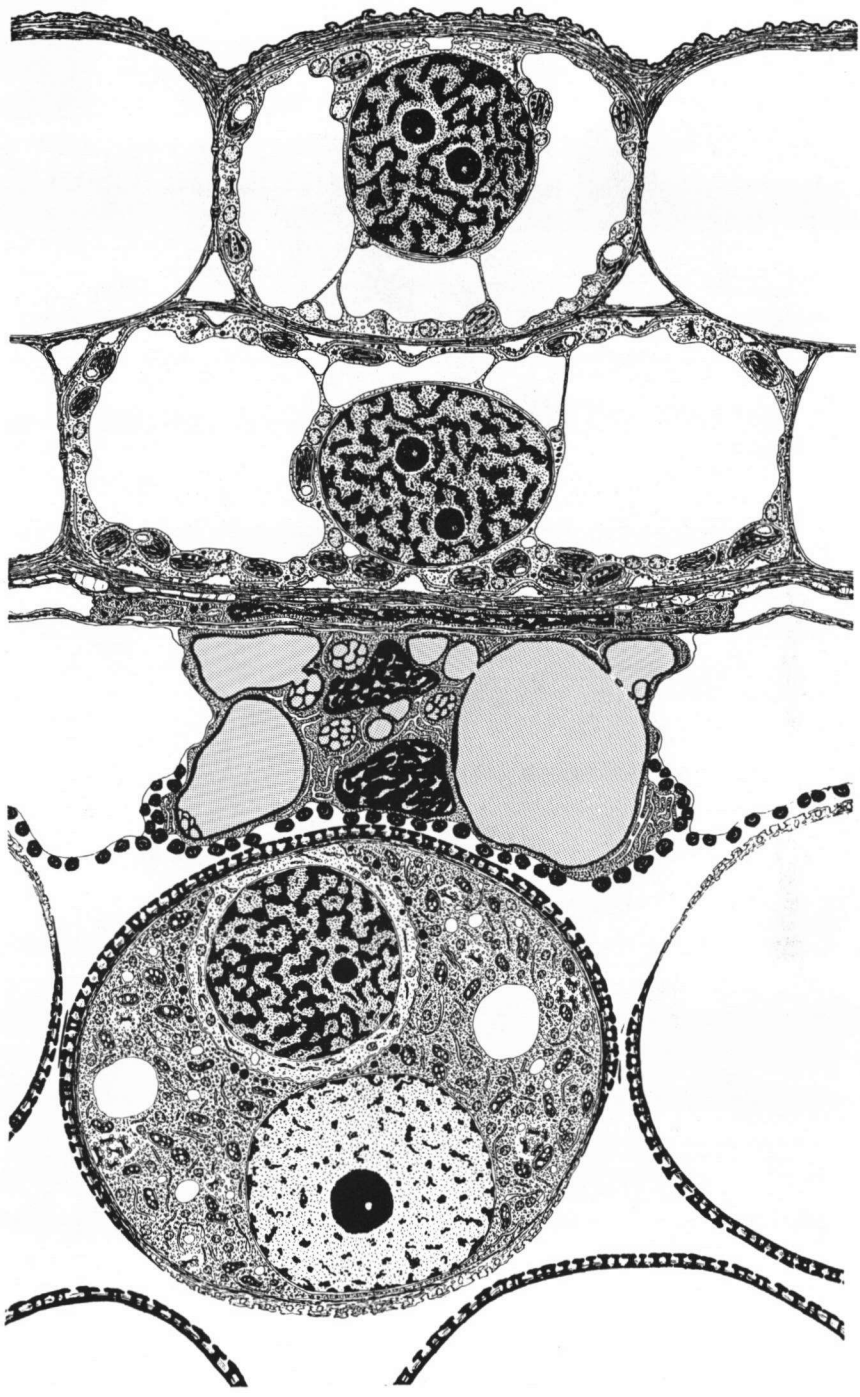
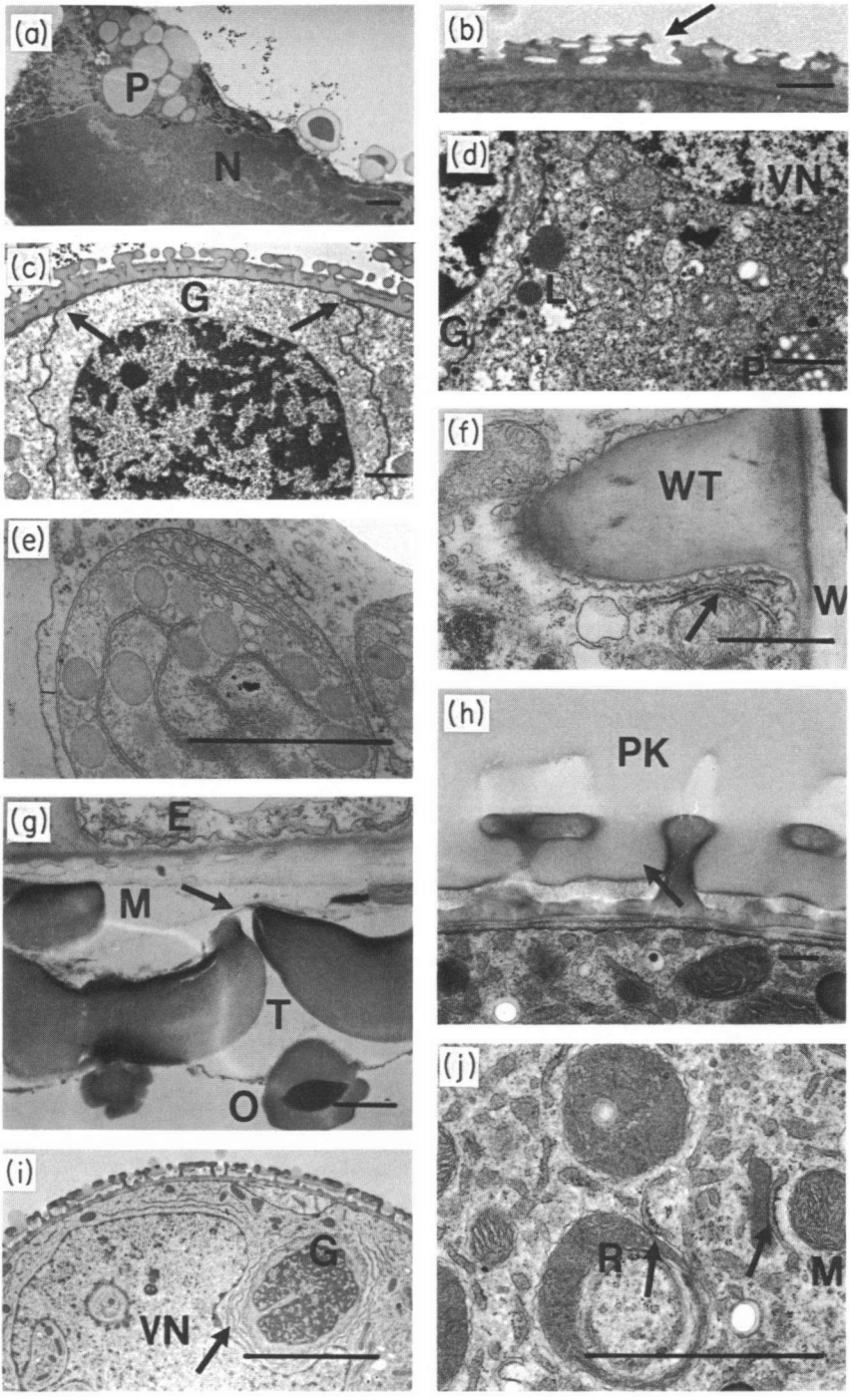


Fig. 5. The generative cell is set free from the intine. Both the epidermis and the endothecium cells swell by vacuolation. The latter forms U-shaped wall thickenings. The cuticular surface is enlarged by the formation of wall ridges. The tapetal degeneration is almost complete, the cell content turns into large drops of pollenkitt. The generative cell has lost its callose wall and is pinched off from the intine. The amount of cytoplasm in the vegetative cell increases contrary to the vacuolation.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The role of the epidermal wall ridges, reported before by Cheng *et al.* (1981) in *Oryza sativa*, Keijzer (1987a) in *Gasteria verrucosa* and Keijzer and Cresti (1987) in *Aloe* spp., is unclear. The accompanying enlargement of the cuticular surface can explain the improved ability to evaporate, demonstrated by Keijzer (1983), which is necessary for dehiscence (Keijzer 1987a). However, the continuously slight thickening of the cuticle contradicts this theory. Moreover, similar structures can be found in the epidermis of non-desiccating floral parts, e.g. pistils and filaments (Willemse & Franssen-Verheijen 1986; Keijzer *et al.* 1987a,b), which may indicate a function in the rigidity of these organs (Keijzer *et al.* 1987b).

The development of the middle layer

The cytoplasm of the middle layer hardly changes during development (Keijzer & Willemse 1988) and, shortly before its degeneration, the amounts of RER, ribosomes and lipids increase. Apart from the continuous tangential stretching of the cells, due to both the enlarging circumference of the locule and the lack of anticlinal divisions (Davis 1966), the final isolation from the endothecium is remarkable. The disappearing plasmodesmata between these two tissues in younger stages (Keijzer & Willemse 1988), followed by the dissociation of the two tissues from each other indicates a deviating development, possibly related to the tapetal development. Although the middle layer degenerates from a later stage, and more quickly than the tapetum, the preceding increase and relatively long persistence of RER, ribosomes and lipids are comparable with the tapetal degeneration. During the transfer of the pollenkitt to the locule the cytoplasmic remnants of the middle layer are mixed with those of the tapetum and so become part of the tryphine (Keijzer 1987b). Both these data suggest that the middle layer contributes to the tapetal production. The latter hypothesis is supported by the undulating plasma membrane in stage 2, probably indicating excretion activity; it is possible that any sporophytic substance can be mixed with the developing pollenkitt. It may also include lytic enzymes for the degradation of its cell walls. The continuation of this digestion after the lysis of most of the cytoplasm of the cell may be caused by the endothecium, which can be seen by its undulating plasma membrane.

A contribution of the middle layer in any tapetal production agrees with the findings of Reznickova and Willemse (1980) for the inner middle layer of *Lilium hybrida*. However, in

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Fig. 6. (a) The tapetal cells are mainly filled with a degenerated nucleus (N), large globules of pollenkitt (P), ribosomes and RER. The same stage as in Fig. 3g–i (bar = 1 μm). (b) In the stretched colpus some of the isolated plasma membrane undulations are in open contact (arrow) with the locular cavity. The same stage as in Fig. 3 g–i and 6a (bar = 1 μm). (c) The contact sites (arrows) of the division wall and the intine approach each other and the total contact surface of the generative cell (G) and the intine decreases. Between the plasma membranes of the vegetative and the generative cell no wall material can be observed (bar = 1 μm). (d) The generative cell (G) is surrounded by lipid droplets (L) in the vegetative cytoplasm. The plastids (P) in the latter contain little starch and electron-dense material. The vegetative nucleus (VN) is heterochromatic. The same stage as in Figs 3g–i and 6a–c (bar = 1 μm). (e) In the mature pollen stage the plastids of the epidermis (and the endothecium) are mainly filled with plastoglobules (bar = 1 μm). (f) In the mature pollen stage the wall (W) between the middle layer and the endothecium is very thin. Inside the latter some RER cisterns (arrow) border the wall thickenings (WT) (bar = 1 μm). (g) In the mature pollen stage the inner tapetal membrane (arrow) borders the endothecium cell (E) since most of the contents of the middle layer (M) has disappeared. A small part of the pollenkitt is still in the tapetum 'cell' (T). O = orbicule (bar = 1 μm). (h) Pollenkitt (PK) fills the exine cavities (arrow) of the mature pollen grains (bar = 0·1 μm). (i) In the mature bicellate pollen grain the lobed vegetative nucleus (VN) and RER stacks (arrow) surround the spindle-shaped generative cell (G), which is shown in cross-section (bar = 100 μm). (j) In the mature pollen grain the mitochondria (M) have swollen cristae and the plastids (P) are bordered by semi-rough ER, the smooth side of the cisterns facing the plastids (arrows) (bar = 1 μm).

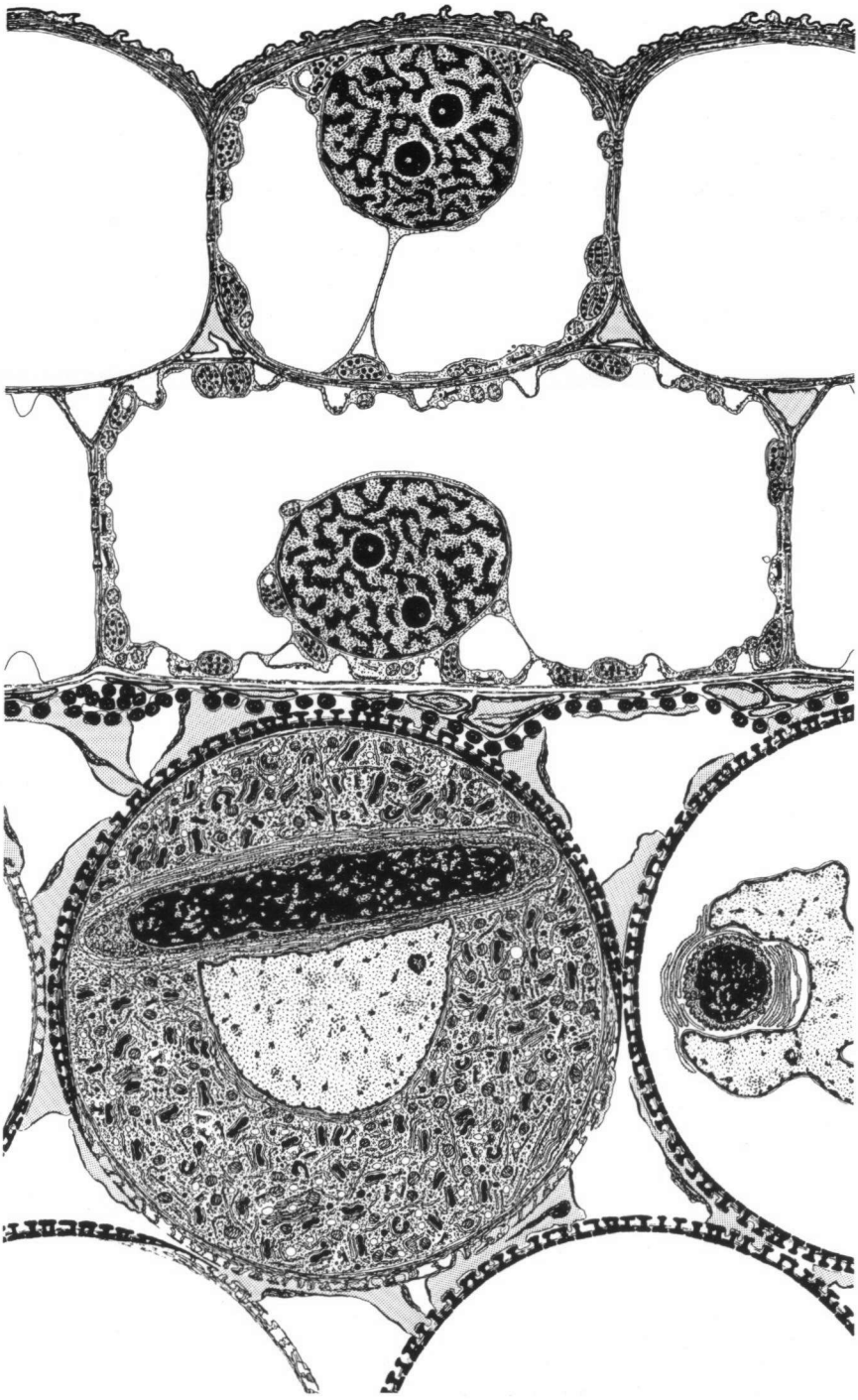

Fig. 7. The mature pollen stage. The epidermis and endothecium cells have reached their maximal volume and bend the locular wall in a centripetal direction due to the endothecial wall thickenings. The pollen grains expand into the tapetum and middle layer cells so that the pollenkitt is sucked into the locular cavity between the pollen grains. The microtubule-rich, spindle-shaped generative cell is enveloped by the vegetative nucleus.

contrast with their findings, any similarity between the plastidial contents of the middle layer and the tapetum is not found in *G. verrucosa.*

The roles of the tapetum

The tapetal functions, reviewed by Pacini *et al.* (1985), can be divided into three main groups (Keijzer & Willemse 1988). Those functions that can be related to our observations will be discussed here.

In earlier developmental stages the most important role of the tapetum is the organization of the cell wall changes (Keijzer & Willemse 1988), after the microspore release cytoplasmic degeneration becomes the most remarkable process in the tapetal cells.

During the young microspore stage the tapetum cells reach their largest size (Keijzer & Willemse 1988) and they then start to degenerate. The starch and lipid gradually disappear and are partly involved in the synthesis of the large amount of pollenkitt (Keijzer 1987b). Mitochondria and dictyosomes disappear after the pollen mitosis, the nucleus degenerates more slowly, while most of the RER and ribosomes remain until the last degeneration stages and are partly deposited on the pollen grains as tryphine. In many species there is a close correlation between this 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, due to the callose breakdown rather than to tapetal supply (Keijzer & Willemse 1988). This amount increases slightly up to the mitotic division. After this division, however, halfway through the tapetal degeneration, the amount of cytoplasm in the vegetative cell increases sharply, while the locular fluid gradually disappears (Keijzer 1983). This might indicate that tapetal breakdown products are stored temporarily in the locular fluid (Pacini & Franchi 1983) and are only transferred to the pollen grains after the cytoplasm of the generative cell has been isolated from the vegetative cell. The persisting tapetal RER and ribosomes indicate a continuous synthesis of proteins, presumably lytic enzymes, for the cell itself and sporophytic pollen wall proteins (Heslop-Harrison *et al.* 1973). However, the incorporation of enzymes into the pollen coating must not be excluded, since RER often borders the developing globules of pollenkitt (Keijzer 1987b), and ribosomes can finally be traced in the tryphine on the pollen grains. The transfer of the final tapetal content, i.e. mainly pollenkitt, and a small amount of tryphine, to both the locule and the intercellular spaces between the epidermis and the endothecium, is a result of capillary forces in the crowded locule (Keijzer 1987a; Keijzer & Cresti 1987).

Apart from possible relationships between disappearing tapetal substances and appearing pollen substances, the apparently normal continuation of the pollen development on rather simple culture media *in vitro* (Tanaka & Ito 1980, 1981; Tanaka *et al.* 1980) indicates a considerable autonomy for many of these processes.

The developing pollen grains

The meaning of polarity. Polarity appears immediately upon the formation of the microspores. The first signs are the centrifugal position of both the nucleus and ER along the plasma membrane of the young tetrad, the ER presumably prevents the deposition of a fully developed exine over the future colpus (Willemse 1972). During the late tetrad stage the nucleus migrates to the wall opposite the colpus by the activity of microtubules (Van

Lammeren *et al.* 1985). After the callose digestion most of the cytoplasm moves to the area between the nucleus and the colpus, surrounded by the rapidly enlarging vacuoles. In this way, the absence of plastids in the generative cell, reported before in this species by Schröder (1985), is an important result of polarity. Furthermore, polarity may play a role in the deviating development of the generative cell. The presence of callose in the wall between the two cells (Keijzer 1987b) is the first indication of a deviating development (Owens & Westmuckett 1983). Moreover, the confluence of vesicles (secundary lysosomes?) with the plasma membrane, near the colpus in the young microspore, suggests that the flow of locular fluid to the pollen grains occurs mainly through the colpus, suggested before by Rowley & Flynn (1971) and Christensen & Horner (1974). Consequently, the generative cell is isolated from the locular fluid, which may effect its deviating development and explains why the callose is restricted to the division wall.

The pollen walls. The exine development of *G. verrucosa* is thoroughly described by Willemse (1972). Apart from a protective function with storage sites for proteins (Knox 1984), its hydrophobic nature plays a role in pollen dispersal (Keijzer 1987b). The elasticity of the exine is mainly restricted to its reduced part on the colpus, which allows a considerable harmomegathy for the cell. In the final developmental stages (3 and 4) this thin sporopollenin layer is disrupted due to stretching of the colpus, by which the pinched off plasma membrane undulations of the intine are opened towards the locule. Since the latter is rather dry just before dehiscence (Keijzer 1983), this will presumably not lead to a loss of the hydrolytic enzymes and recognition substances which are stored in these sites (Knox 1984). The latter substances may originate from the ER which borders these plasma membrane undulations during their formation in the vacuolated microspore.

The development of the generative cell. Soon after its deposition the generative cells is tied off from the intine, by which its callose wall disappears (Keijzer 1987b). An association between this process and microtubule activity (Dickinson 1975) has not been found in *G. verrucosa* until now (Van Lammeren *et al.* 1985). In contrast, the preferential presence of RER stacks near the edge of the generative cell in stage 2 indicates a role for these structures in the disjunction. Their initial position around the nucleus has, presumably, to do with their formation. In the mature pollen grain comparable stacks appear along the entire surface of the generative cell, which makes their role in the storage of substances for germination (Jensen *et al.* 1974; Cresti *et al.* 1985) in *G. verrucosa* less likely.

The final spindle-shape of the free generative cell is maintained by many microtubules parallel to its longitudinal axis (Van Lammeren *et al.* 1985); a general feature (Sanger & Jackson 1971). The close association of the generative cell with the vegetative nucleus, also a general feature (Wilms *et al.* 1986), remains after pollen dehydration (Keijzer *et al.* 1986; Wilms *et al.* 1986) and may serve as a transport configuration.

The reserve substances in the mature pollen grain. Although starch is still present, the main reserve substance in the mature pollen grain of *G. verrucosa* is lipid, which agrees with the classification of Baker & Baker (1979) for *Liliaceae*. After the mitotic division the plastids gradually lose most of their starch and turn into an increasing number of elaioplasts, most

of them containing a small starch grain. Quantitative data of the changing plastid population of the developing pollen grain in this species are reported by Willemse (1972). Concomitantly, the amount of cytoplasmic lipid droplets increases, initially only around the dissociating generative cell, but later on dispersed throughout the entire vegetative cell. This might indicate that the lipids have three possible sources: firstly, the callose from the generative cell wall; secondly, the starch and thirdly, soluble precursors from the degenerating tapetum, transported via the locular fluid. Apart from these lipid reserves, the ER that lines the plastids at maturity indicates the future release of plastidial reserves (partly starch) during pollen tube growth (Cresti & Keijzer 1985).

CONCLUSIONS

Conclusions can be drawn about the entire locule development from the results of this paper and the preceding report on this subject (Keijzer & Willemse 1988, this issue).

The ultrastructural changes in the epidermis and the endothecium are strongly comparable and can be associated with the dehiscence process. Roles of these tissues in the pollen development can probably better be traced with labelling experiments (Reznickova 1983).

Although the development of the middle layer indicates some similarities with the tapetum, its cytological changes are few, while its degeneration starts too late to given an important supply of breakdown products to the developing pollen. An exception may be the presence of cytoplasmic remnants in the tryphine.

The sequence of cell wall digestions (Keijzer & Willemse 1988), the tapetal degeneration, the locular fluid disappearance and the pollen development suggest a shift of carbohydrates from the original meiocyte walls to the tapetum, followed by an opposite transfer from the tapetum to the developing pollen grains, by which the locular fluid probably serves as a temporary storage site. A final function of the tapetum is the synthesis of pollenkitt, apart from (sporophytic) proteins, as reviewed by Knox (1984).

Different interactions between the investigated anther tissues can be found from this and earlier studies in this species. The collaboration between the epidermis and the endothecium seems to be rather clear and mainly supports the dehiscence process (Keijzer 1987a). Interactions between the middle layer and the bordering tissues are difficult to demonstrate, in contrast with the results of Reznickova & Willemse (1980) in *Lilium hybrida*. In our opinion, interactions between the tapetum and the developing pollen grains occur in two directions. Apart from the often described transfer of substances from the tapetum to the pollen grains, the meiocytes influence the tapetal development with the offer of derivatives from their original walls. In later stages a directing role of the pollen grains in the tapetal development cannot be demonstrated and is probably absent (Keijzer & Cresti 1987). However, the presence of the pollen grains is indispensable for the transfer of the pollenkitt from the tapetum towards the pollen grains (Keijzer 1987b; Keijzer & Cresti 1987).

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