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The ovule of *Gasteria verrucosa* at receptivity of the stigma: an ultrastructural study

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SUMMARY

An ultrastructural study on the embryo sac and the micropylar part of the nucellus, at the time of stigma receptivity, was carried out to reveal possible changes due to pollination. At the time of stigma receptivity, the development of the egg cell, central cell, antipodals and the micropylar nucellar cells of *Gasteria* have been completed. Only the synergids and the filiform apparatus are still differentiating. Frequently, anomalies in the morphology of the unfertilized embryo sac are observed. Comparative observations before and 2·5 h after cross-pollination did not show ultrastructural changes in the embryo sac and in the micropylar nucellar cells. The egg cell showed polarity. The nucleus and most of the cytoplasm lay in the micropylar half, while vacuoles filled most of the chalazal half. The two synergids did not differ from each other and a clear degeneration had not taken place. Based on the observed ultrastructural differences of the filiform apparatus and the synergid cytoplasm among the investigated ovules, and data of previous studies, a sequence of differentiation of the very large filiform apparatus was defined. The nucleus and most of the cytoplasm of the central cell were positioned near the degenerating antipodals. The ultrastructure of the micropylar nucellar cells indicates a secretory function of these cells and an involvement in the process of pollen tube acceptance.

Key-words: Gasteria, nucellus, ovule, receptive embryo sac, secretion, synergid.

INTRODUCTION

The ovary of a pollinated *Petunia* flower changes its protein synthesis about 18 h before the pollen-tube tips reach the ovary (Deurenberg 1976, 1977). This indicates a long-distance transmission of information from stigma or style to the ovary ahead of the pollen tubes. Earlier studies on *Gasteria* have shown some ultrastructural changes in the stylar canal cells and ovular papillar cells 30 min after pollination, i.e. when the pollen tubes have grown into the stigma only (Willemse & Franssen-Verheijen 1986). Two-and-a-half hours after cross-pollination, the pollen tubes have grown approximately 2 mm into the style, and after another 2 h they reach the top of the ovary (Sears 1937; Willemse & Franssen-Verheijen 1988). Because of the short duration of the pollen-tube growth in *Gasteria*, the

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transmission of a possible stimulus, if any, must be very quick. In this study, the embryo sac and the micropylar part of the nucellus were observed 2·5 h after cross-pollination, in order to determine whether ultrastructural changes occur in relation to pollination. Previous ultrastructural studies have shown the development from the megaspore mother cell to the young embryo sac of *Gasteria*, before anthesis, to be of the Polygonum type (Willemse & Franssen-Verheijen 1978).

MATERIALS AND METHODS

Gasteria verrucosa (Mill.) H. Duval plants, which exceptionally produce seeds after self-pollination, were grown in a glasshouse. One day before anthesis, the flowers were emasculated. When a droplet of exudate was visible on the stigma (approximately 4–5 days after anthesis), slices of the central part of the ovary were fixed in 3% glutaraldehyde in 0·1 M phosphate buffer, pH 7·2, for 18 h at 4°C. After washing in the same buffer the tissue was post-fixed in 1% OsO_4 in the same buffer for 2 h at 20°C. After washing and dehydration in an ethanol series, the tissue was embedded in Epon. The same treatment was carried out with comparable slices of the ovary, 2·5 h after cross-pollination. Longitudinal serial sections of ovules were studied and photographed with a Philips 301 TEM. Callose was traced by staining fresh sections of the ovary with aniline blue. These sections were studied with UV-fluorescence microscopy.

RESULTS

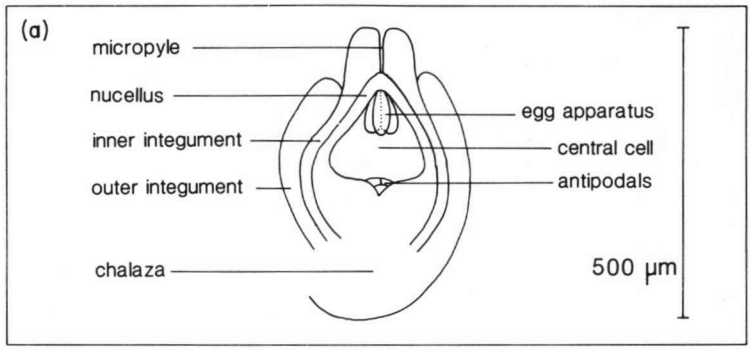
In comparative observations of nine ovules from pollinated and unpollinated flowers at the receptive stage of the stigma, differences in ultrastructural morphology due to pollination could not be observed. A survey of an ovule and the results of the ultrastructural analysis of the different cells of the embryo sac and the micropylar nucellar cells are represented schematically in Fig. 1. The most noteworthy events concerning the different cells are described below.

Egg cell

The egg cell is enclosed by a flat plasma membrane and a wall that is only present at the micropylar side. Plasmodesmata can be seen in the walls facing the synergid cells and the central cell. The round nucleus is situated in electron-dense ground plasma at about a third of the cell length from the micropylar pole of the cell. Most of the cytoplasm is situated around the nucleus and in the micropylar part of the cell. Accordingly, most vacuoles are seen in the chalazal part and some smaller ones in the micropylar part. In comparison with the synergids and the central cell, there are many, often elongated, mitochondria and only a few dictyosomes (Fig. 2a). The microtubules are positioned cortically.

Synergids

A clear distinction in morphology and ultrastructure was not observed between the two synergids. The lobed nuclei are situated in the chalazal part of the cells against the filiform apparatus and close to the plasma membrane facing the central cell. In the electron-dense ground plasma, microtubules are seen around the nuclei as well as in the cortical cytoplasm (Fig. 2b). The many dictyosomes show some large but faintly visible vesicles. The RER shows large stacks of dilated cisternae (Fig. 2c) and is situated predominantly in



(b)	synergids	egg cell	central cell	antipodals	micropylar nucellar cells
cell morphology					
nucleus					
nucleolus					
nuclear envelope					
microtubules					
ribosomes					
ER					
vacuoles					
vesicles					
dictyosomes					
plasma membrane					
mitochondria					
plastids					
spherosomes					
ground plasm					

Fig. 1. (a) Schematic drawing of a median longitudinal section of an ovule of *Gasteria*. (b) Schematic representation of the ultrastructural analysis of the different cells of the embryo sac and the micropylar nucellar cells from six ovules of *Gasteria* at stigma receptivity. For the cell morphology the dotted lines represent the vacuoles. The frequency of symbols of the vesicles, dictyosomes and spherosomes represents the estimated amount. For the mitochondria, the morphology and their estimated amount are represented by the small symbols. ER = endoplasmic reticulum.

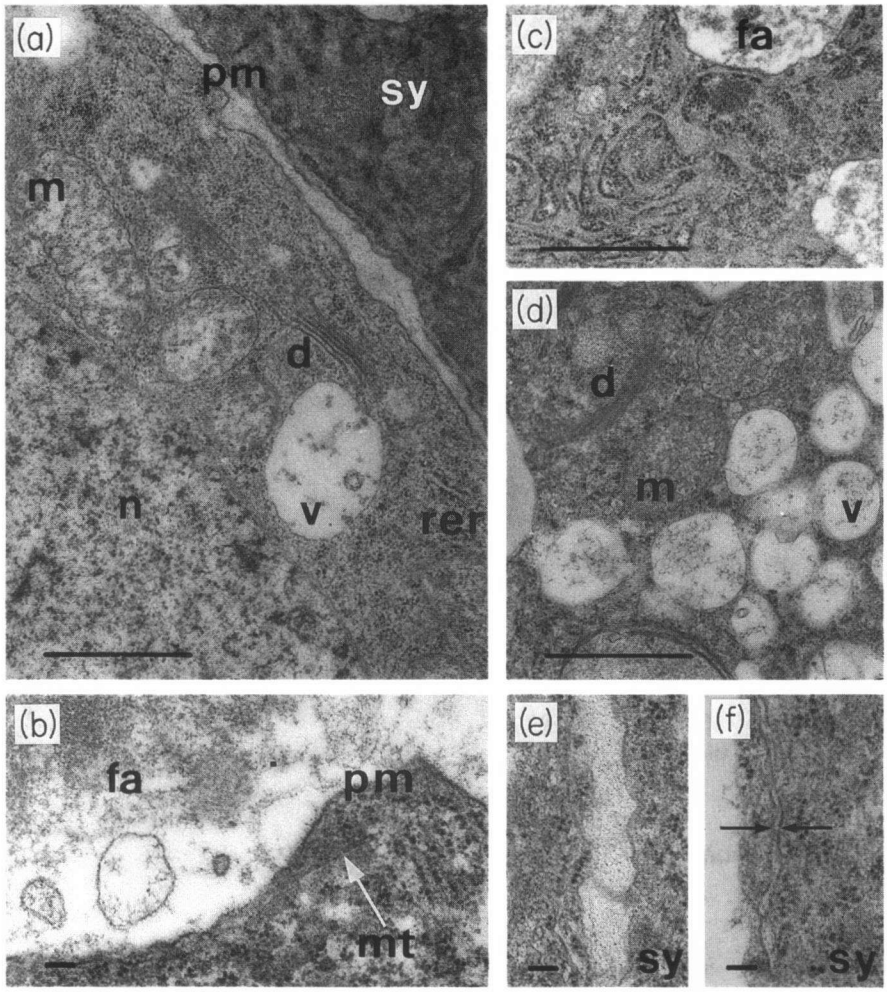


Fig. 2. (a) Part of the egg cell of *Gasteria* at stigma receptivity showing organelles in the electron-dense ground plasma. × 19,400. (b) Filiform apparatus with vesiculate zone and the plasma membrane of the bordering synergid of *Gasteria*. × 35,300. (c) Dilating rough endoplasmic reticulum in a synergid near the filiform apparatus of *Gasteria* during its early differentiation. × 19,400. (d) Cytoplasm of chalazal part of a synergid near the filiform apparatus of *Gasteria* during its early differentiation, showing vesicles with a fibrillar content in the electron-dense ground plasma. × 19,400. (e) Cell wall with plasmodesmata between the central cell and synergid at the micropylar side of the egg apparatus of *Gasteria*. × 35,300. (f) Plasma membranes (arrows) of the central cell and the synergid at the chalazal side of the egg apparatus of *Gasteria*. × 35,300. Abbreviations: c, channel; cc, central cell; cd, curved dictyosome cisterns; cz, compact zone; d, dictyosome; f2, fibrillar zone; fa, filiform apparatus; hz, heteromorphic zone; i, inner integument; m, mitochondrium; mt, microtubules; n, nucleus; nc, nucellus; p, plastid; pm, plasma membrane; rer, rough endoplasmic reticulum; s, spherosome; sy, synergid; t, tonoplast; tz, transparent zone; v, vesicle; vz, vesicilate zone; w1, cell wall near embryo sac; w2, cell wall between two nucellar cells. The scale bars represent 1 μm.

the chalazal part of the cell. Also in this part many vesicles with a fibrillar content can be seen (Fig. 2d). The plasma membrane is almost straight, except opposite the filiform apparatus, where it meanders. At the micropylar side, cell walls with plasmodesmata are present (Fig. 2e), towards the chalazal side they are absent (Fig. 2f). The filiform

apparatus is very large and occupies the main part of the cells: its tip reacts positively for callose (Fig. 4a).

The ultrastructure of the filiform apparatus, the nuclei, RER and dictyosomes and the number of vesicles and spherosomes vary. Based on data from the young embryo sac before anthesis (Willemse & Franssen-Verheijen 1978) and the observations in this study on embryo sacs at the time of stigma receptivity, 4–5 days after anthesis, five developmental phases of the filiform apparatus and the synergids can be distinguished, i.e. within the period of stigma receptivity an ultrastructural differentiation is observed that is not affected by pollination. These phases are represented schematically in Fig. 4b. The filiform apparatus shows a central heteromorphic zone (Fig. 6b), containing cytoplasmic remnants surrounded by a fibrillar zone of electron-translucent and fibrillar material (Fig. 3a). The fibrillar zone gradually develops into an inner, more electron-dense, compact zone and an outer vesiculate zone. During these developmental phases the stacks of RER become less dilated, the numbers of vesicles and spherosomes increase (Fig. 3b) and curved dictyosome cisterns are found. In the last phase of differentiation observed, the outer zone of the filiform apparatus consists only of a very thin electron-transparant layer (Fig. 3c). In this stage the RER and spherosomes are less in number and both nuclei are degenerating. Degeneration of the cytoplasm of one of the two synergids was never observed. If the synergids had both degenerated, the whole egg apparatus had been degenerated.

Central cell

The main part of the cell is occupied by a central vacuole with a fibrillar content. Most of the cytoplasm is situated around the large nucleus, which lies near the antipodals. Microtubules are seen cortically and around the nucleus in a parallel arrangement to each other; around the nucleus microtubules can be seen, with random orientation. The other organelles are regularly distributed in the slight electron-dense ground plasma. The RER shows small stacks of large, sometimes dilated, cisterns and a high number of dictyosomes, with many vesicles, are present (Fig. 4c). Except for opposite the chalazal part of the egg apparatus, a cell wall with plasmodesmata to the other cells of the embryo sac is present (Fig. 2e, f).

Antipodals

The small antipodal cells are situated in a depression at the chalazal end of the embryo sac. Their plasma membrane undulates, and they are surrounded by walls. Plasmodesmata are found in all walls, including those facing the nucellar cells. Sometimes cortically situated microtubules are found. In the centrally positioned nucleus, nucleoli are absent. The plastids show hypertrophy and are difficult to distinguish from vacuoles (Fig. 5).

Micropylar nucellar cells

At the micropylar side, one layer of nucellar cells borders the embryo sac. In these cells a round centrally positioned nucleus with one or two electron-dense nucleoli is surrounded by large vacuoles. In the direction of the chalaza the vacuoles are smaller. The plasma membrane undulates and shows lomasomes and intrusions in the bordering vacuoles. In the slight electron-dense ground plasma cortically situated microtubules are observed. The often elongated mitochondria differ from those in the embryo sac, having many swollen cristae. The cell wall facing the embryo sac is thick. The remnants of the earlier degenerated nucellar cells lie between this cell wall and the wall of the embryo sac (Fig. 6a).

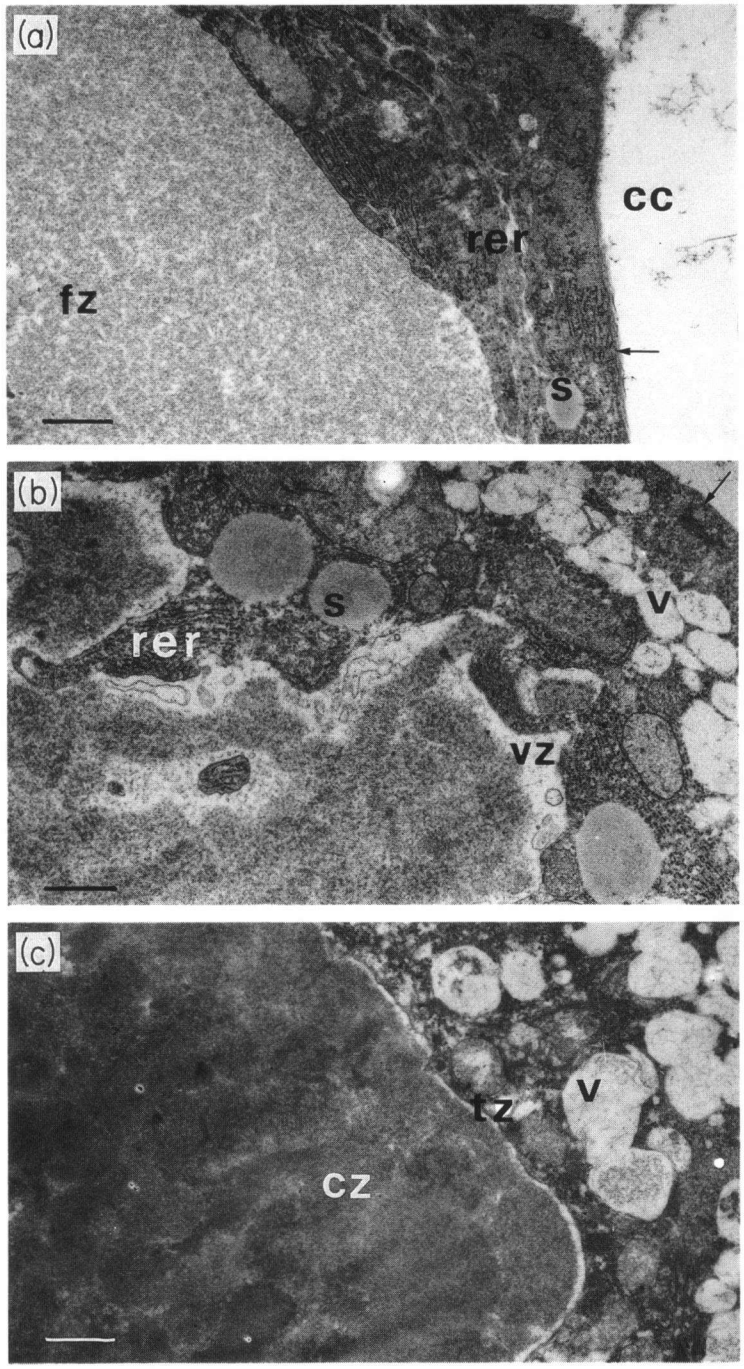


Fig. 3. Basal parts of the filiform apparatus and bordering synergid cytoplasm at three stages of differentiation during stigma receptivity of *Gasteria*, in order of differentiation. Arrow: plasma membranes of synergid and central cell. × 9,200. Abbreviations as for Fig. 2. Scale bar represents 1 μm.

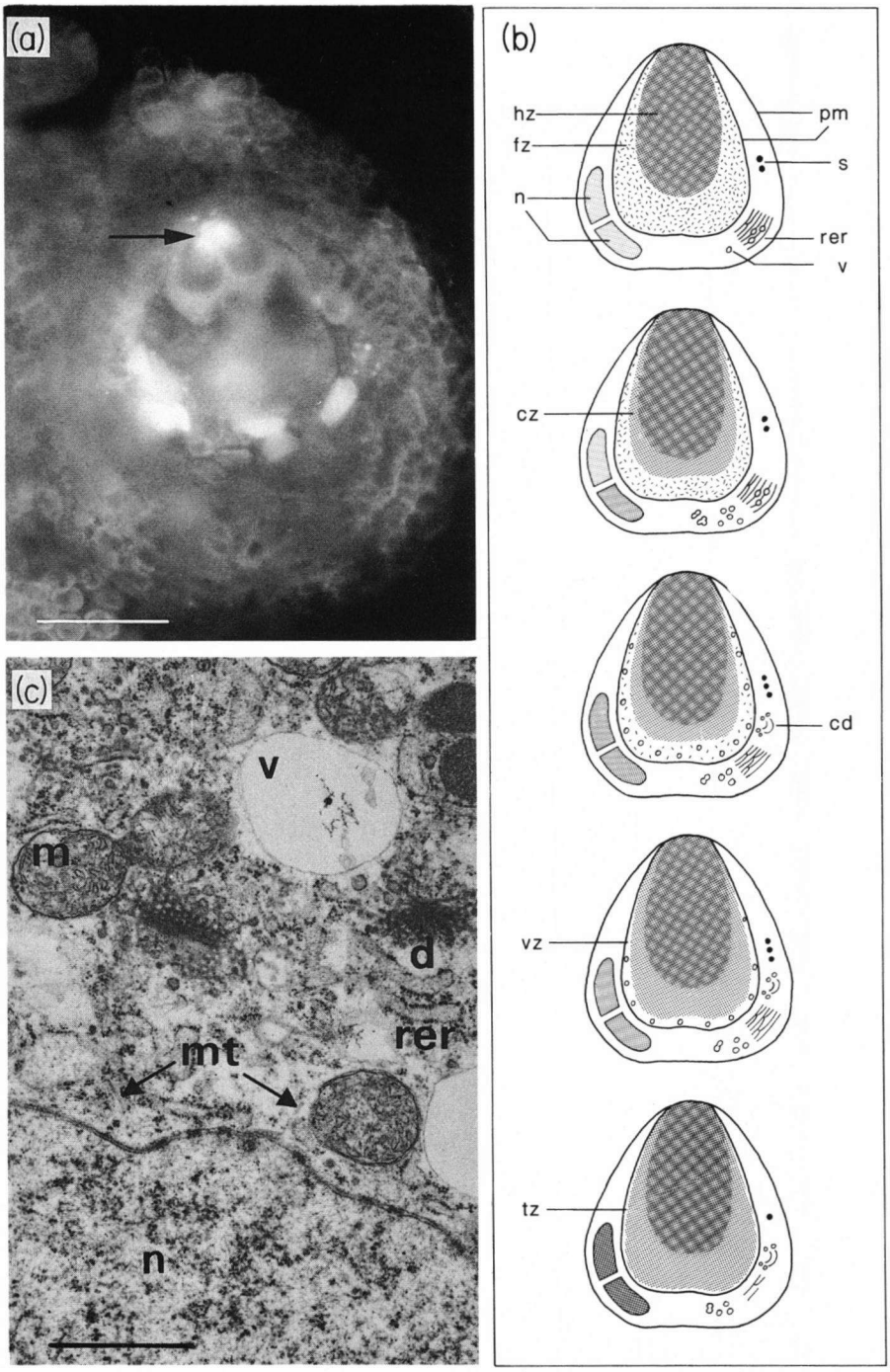


Fig. 4. (a) Fresh longitudinal section of the ovule of *Gasteria*, at stigma receptivity, stained for callose. × 175. Arrow indicates the callose in the top of the filiform apparatus. Scale bar represents 100 μm. (b) Schematic representation of the most remarkable differences between five successive phases of differentiation of a synergid and its filiform apparatus during the period of stigma receptivity of *Gasteria*. The inner plasma membrane surrounds the filiform apparatus and the two drawn nuclei represent the nuclei of both synergids of one embryo sac. (c) Part of the central cell of *Gasteria* at stigma receptivity. × 19,400. Note the dictyosomes with many vesicles in the slight electron-dense ground plasma. Scale bar represents 1 μm. Abbreviations as for Fig. 2.

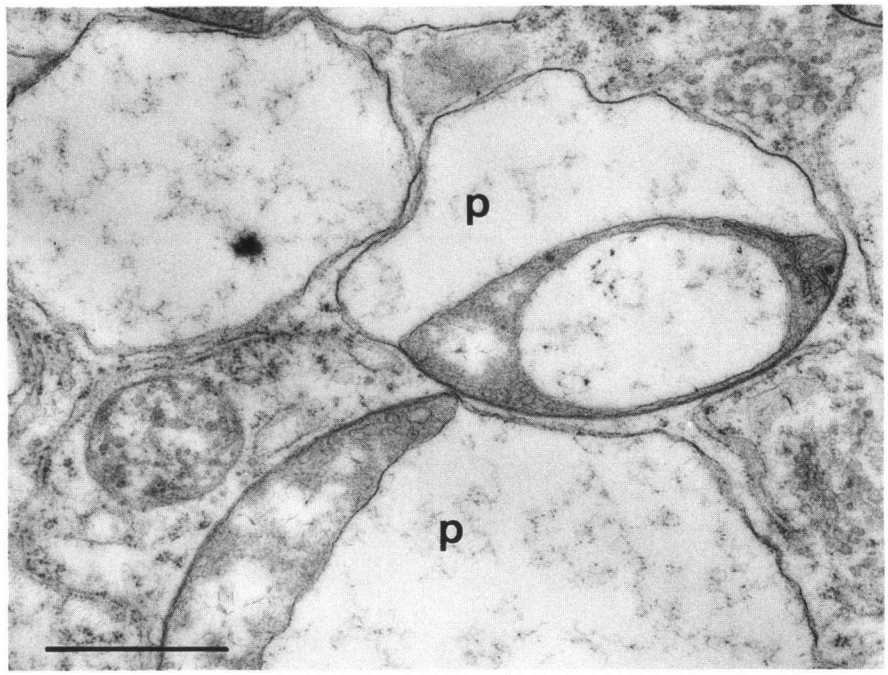


Fig. 5. Hypertrophied plastids in the antipodals of *Gasteria* at stigma receptivity. × 23,800. Scale bar represents 1 μm. Abbreviations as for Fig. 2.

Plasmodesmata interconnect the nucellar cells. The wall near the micropyle has lost its cuticle. Remnants of this cuticle and other electron-dense material are found between the extreme top of the nucellus and the inner integument. At this side two neighbouring nucellar cells are separated by a channel that is also filled with electron-dense material (Fig. 6b).

In some of the investigated ovules, aberrant embryo sacs were found. Some embryo sacs showed an incomplete development. An extreme case concerned an embryo sac with only fragments of the egg apparatus and an enlarged central cell with one nucleus and with walls in its micropylar part. In this case the micropylar nucellar cells protruded out of the micropyle and were dividing. The stigma of this flower was not pollinated.

DISCUSSION

The structure and development of the embryo sac and the nucellus of *Gasteria* shows a close similarity with *Ornithogalum* (Willemse & Franssen-Verheijen 1978; Tilton & Lersten 1981; Tilton 1981). During the receptive stage of the stigma of both pollinated and unpollinated flowers, the ultrastructure of the egg cell, central cell, antipodals and the micropylar nucellar cells remained almost the same. Only the rate of differentiation of the synergids and their filiform apparatus varied between investigated ovules.

In the filiform apparatus, an electron-transparent zone containing vesicles was observed, as in *Ornithogalum* (Tilton 1981). In the cytoplasm the dilation of RER cisterns diminished during the last phase of differentiation. Near the border of the filiform apparatus the outer zone with vesicles converted into a more dense structure without

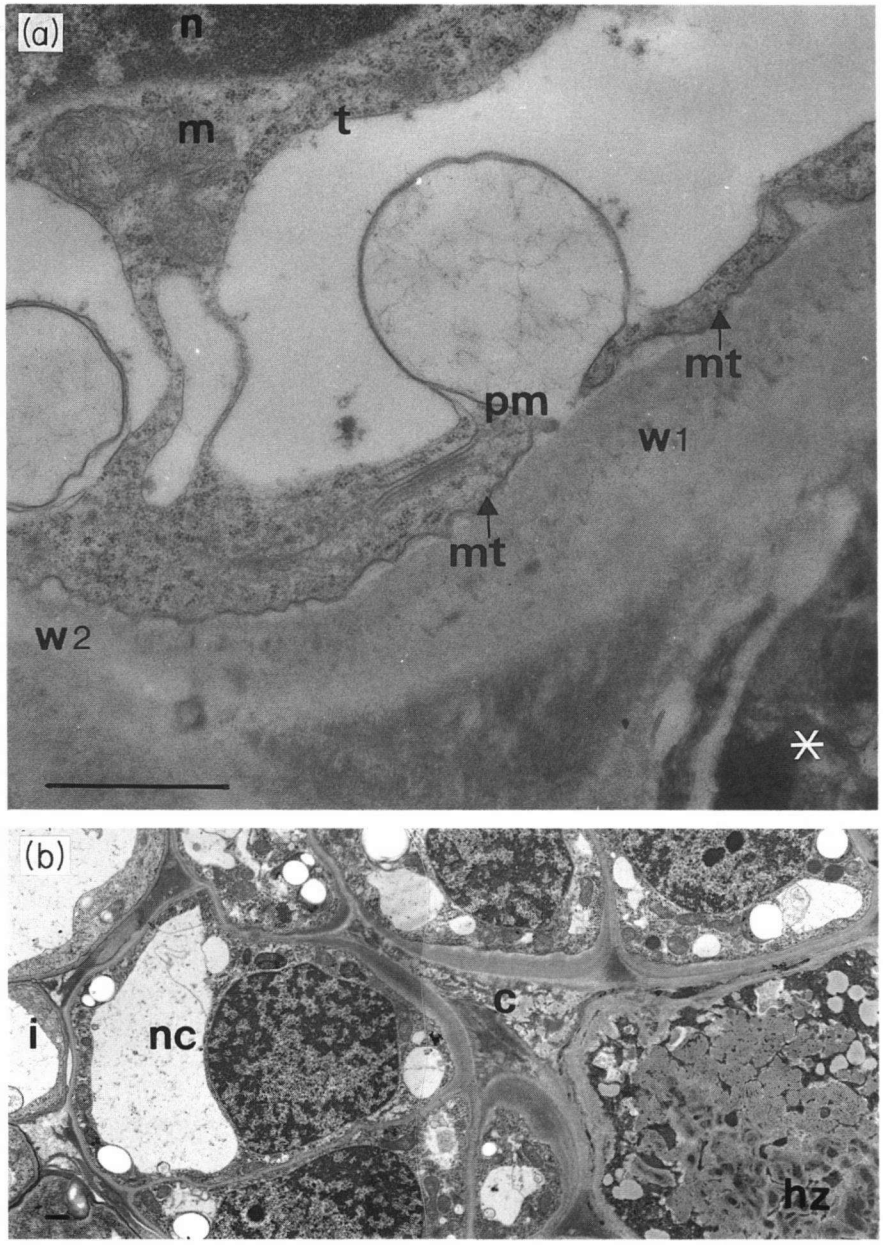


Fig. 6. (a) Part of a micropylar nucellar cell of *Gasteria* with intrusions of the plasma membrane into a vacuole; *: degenerated nucellar cells. × 23,800. (b) Longitudinal section of the top of the embryo sac with the neighbouring nucellar cells of *Gasteria* at stigma receptivity. Note the content of micropylar channel. × 2,800. Scale bars represent 1 μm. Abbreviations as for Fig. 2.

vesicles. Only a small electron-transparent zone remained. In the cytoplasm the stacking of RER decreased. The vesiculate stage seemed to precede the more solid stage of the filiform apparatus. The vesicles, present in the synergid cytoplasm, are supposed to be related to a lysosomal function to facilitate the passage of the sperm cells through the

protoplast of the synergid (Secor & Russell 1988). However, in *Gasteria*, degeneration of only one of the synergids was not observed during the receptive period. Where both synergids had degenerated, the egg cell was also degenerating.

The functions of the synergids have been discussed in several reports (Vijayaragha van & Bhat 1983; Willemse & Van Went 1984). They are supposed to be active in the synthesis and secretion of substances related to pollen-tube growth (Tilton 1981). In *Gasteria*, the tip of the filiform apparatus shows a positive reaction for callose. The substances that are either secreted or absorbed by the synergids have to pass the callosic layer. This suggests the possible function of this layer as a molecular sieve, as hypothesized by Dumas & Knox (1983). The micropylar channel in the nucellus may be formed by dissolution of the middle lamella due to secreted enzymes from the synergids, according to observations of Wilms (1981) in *Spinacia*. Its heterogeneous contents may be the remnants of earlier degenerated nucellar cells. However, it may also consist of excretion products from the synergids or from the nucellar cells and the inner integrument cells lining the micropyle. In *Ornithogalum* these nucellar and inner integument cells already secrete products before anthesis (Tilton & Lersten 1981). Moreover, transfer of products from the placental locule to the embryo sac could be possible; the ovular papillar cells, situated on the placenta between the ovules, have transfer-like walls (Willemse & Franssen-Verheijen 1986), which suggest a secretory function for these cells too.

The antipodals start to degenerate at athesis. The first sign of degeneration is marked by a more condensed nucleus, appearance of vesicles and vacuoles and dilation of the thylakoids (Willemse & Kapil 1981). At the receptive stage of the stigma, degeneration continues; the plastids show a strong hypertrophy of the distance between its outer and inner membrane and the cells contain larger vacuoles.

Because of the ultrastructural variation between synergids of different ovules at the time of stigma receptivity, it is not possible to define the morphological criteria of a normal receptive embryo sac. The presence of exudate does not apparently imply that all the embryo sacs are ready to be fertilized. A further study of fertilized embryo sacs of *Gasteria* will give more information about the relationship between receptivity and ultrastructure.

In the style and ovular papillar cells of *Gasteria*, some ultrastructural changes occur after pollination (Willemse & Franssen-Verheijen 1986). In contrast, 2·5 h after cross-pollination, ultrastructural changes caused by pollination are neither observed in the embryo sac nor in the micropylar nucellar cells. Considering the stability in the ribosome–polysome pattern, an induction of protein synthesis after cross-pollination, as found in the ovary of *Petunia* (Deurenberg 1976, 1977), could not be demonstrated in *Gasteria*.

The nucellar cells show similarities with the stylar canal cells of *Gasteria* described in a previous paper (Willemse & Franssen-Verheijen 1986). The strongly undulating plasma membranes, the presence of lomasomes and mitochondria with swollen cristae and the absence of a complete cuticle may indicate a secretory function, although the dictyosomes are not active in producing vesicles. These data, together with the specific development of the two nucellar top cells bordering the micropyle, indicate that the micropylar nucellar cells are involved in the process of the pollen-tube acceptance.

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