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STYLAR DEVELOPMENT IN THE OPEN FLOWER OF GASTERIA VERRUCOSA (MILL.) H. DUVAL

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SUMMARY

In the style of *Gasteria* changes occur during the five days after anthesis. The stigma becomes receptive for pollen at the time that the perianth starts wilting. The top of the style has a very narrow canal, surrounded by the thick walled papillar and inner epidermal cells. This canal enlarges basipetally and contains a viscous exudate. During the development, the exudate gets osmiophilic granules which disappear when the pollen tube passes.

The cells at the top of the style show some structural differences compared with the other stylar cells, especially in their vacuolation, secretion and number of mitochondrial cristae. Slight cellular changes occur during the passing of the pollen tubes. The other stylar cells seem to have mainly a secretory activity. The papillar cells along the ovules have a transfer cell character. The hollow style of the succulent *Gasteria* may be considered to be a functional solid style with a gradient in the cell wall of the transmitting tissue from solid at the top to fluid at the base.

1. INTRODUCTION

As found in some other Liliaceae *Gasteria* has a wet stigma (HESLOP-HARRISON & SHIVANNA 1977) and a hollow style. Especially the hollow style of several *Lilium* species has been frequently studied. Its papillar stigma becomes wet by the exudate excreted by the papillar transfer cells along the canal (ROSEN & THOMAS 1970; DASHEK et al. 1971; YAMADA 1974). In the canal stigmatoid cells, the precursor for the exudate is probably a protein crystal (GAWLIK 1984). In *Ornithogalum* the stigma papillae show wall ingrowths and secrete exudate with lipids, proteins and carbohydrates. At the base of the funiculi and at the top of the carpel margin, an obturator, consisting of secretory cells, functions as a pathway for the pollen tubes (TILTON & HORNER 1980).

The flower of *Gasteria* is proterandrous, and prior to stigmatic cell enlargement the style elongates and the stigma becomes receptive (SEARS 1937). In this study the ultrastructural changes during stylar development are related to the receptivity, pollination, and pollen tube growth.

2. MATERIALS AND METHODS

Plants of *Gasteria verrucosa* (Mill.) H. Duval were grown in a greenhouse at 18–23 °C, about 60% r.h. and a long day regime of 16 hrs light and 8 hrs darkness. At different stages of the style development small pieces of distinct parts of the

style and of the middle part of the ovary were collected. The different stages are the latest closed flower of a spike, an open flower with an elongated style without exudate on the stigma, a wilting flower with exudate on the stigma unpollinated or cross pollinated by hand.

For electron microscopy the specimens were fixed in 0.1 M phosphate buffer, pH 7.2, with 3.0% glutaraldehyde for 18 hrs at 0 °C. Subsequently the specimens were rinsed with the same buffer and fixed for 2 hrs in 1% 0.1 M buffered OsO_4. After dehydration with alcohol the specimens were embedded in Epon 812. The sections were stained with lead critrate and uranyl acetate and examined with a Philips EM 301 at 60 kV.

2.1. Development and structure of the style

The hollow style of *Gasteria* originates from three carpels and just before the opening of the flower shows a slightly curved, slightly conical stalk of about 10 mm length with a papilate stigma. The stylar canal is three lobed, filled with a viscous fluid: the exudate. At the top of the style the canal is nearly closed (*fig. 1A*).

After anthesis, the style elongates during a perod of about 4 days till the wilting of the perianth. As shown in *fig. 2*, this elongation takes place over the whole length of the style with exception of the upper part (about 2 mm). In the open flower, about 5 mm above the ovary, there is a slight flexion point in the style. During stylar elongation a second flexion point develops about 1 mm below the stigma. In wilted flowers, the style is bended at the first point to nearly a right angle. Both flexions are due to unilateral cell stretching.

About 5 days after anthesis a small droplet of exudate can be observed on the stigma. At that moment the stigma protrudes out of the wilting perianth and pollen can germinate. The compatible pollen tube takes 4 to 5 hours to reach the ovary (see *fig. 2*). After about two days the stigma wilts.

Correlated with the stylar elongation and the appearence of the exudate, the following periods can be distinguished: 1. the latest closed flower period (C); 2. the style elongation period in the open flower (E); 3. the mature style period with exudate on the stigma (M); and 4. the mature style period with exudate on the stigma (ME). During this last period pollen can germinate (P), see *fig. 2*.

The papillar stigma cells of *Gasteria* have a thick wall with a thick undulated cuticle.

Near the stigma, the central part of the style is almost completely filled with solid wall material, covered by a thick cuticle. In the style of the latest closed

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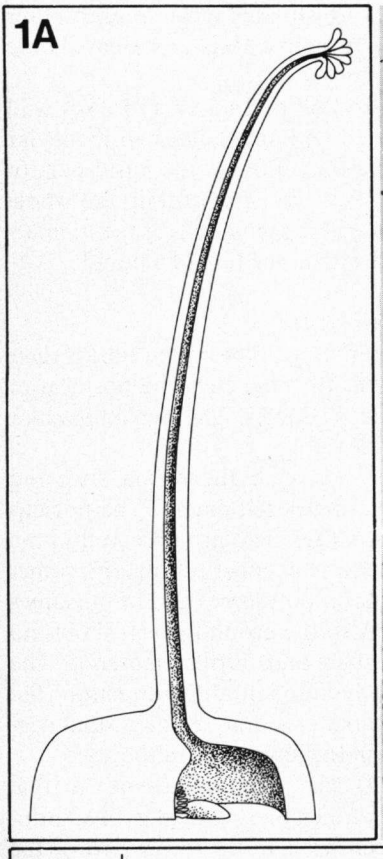
Fig. 1. A. Schematic drawing of the style of *Gasteria*. B. Longitudinal section of the upper part of the style. × 170. C. Transverse section of the stylar middle part. × 170.

Fig. 2. Elongation of a style of *Gasteria* during the open flower period measured at three levels: the top of the stigma; ± 2 mm below the top and in the middle of the style. The base of the style is at the level of the horizontal axis. The dotted line represents the pollen tube length. Stages: C = latest closed flower period; E = style elongation period; M = mature style period; ME = mature style period with exudate.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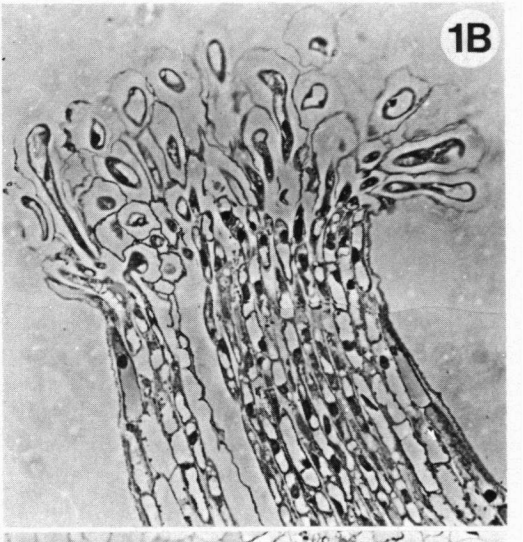
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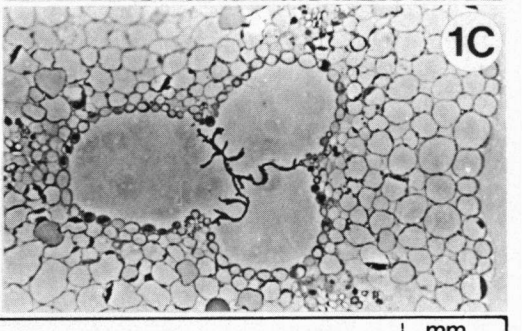
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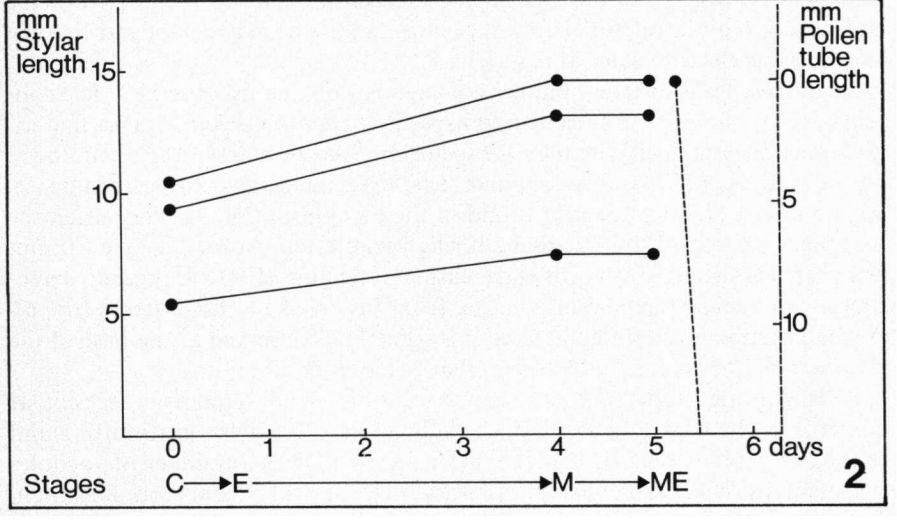
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Stages
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2

flower the cuticles of the inner epidermis cells are situated close to each other and only three narrow canals are left. In the receptive stage these canals are almost closed (*fig. 1B*).

In the lower part of the style the cuticles are thin and cover a viscous wall and during the receptive stage they are ruptured and are situated in the stylar canal (*fig. 1C*). During the development in the open flower, the basal part of the stylar canal is filled with a viscous liquid. This liquid will fill up the whole canal and ultimately it appears in between the papillar stigma cells as the stigmatic exudate. The ovary papillar cells are covered with a fine film of a liquid.

2.2. Subcellular structures in developing styles

A general characteristic of the cells along the pathway of the pollen tube is their elongation in the direction of the stylar length. In these cells the position of the nucleus is nearly central with on both sides vacuoles. The ovular papillar cells are situated lateral to and between the ovules.

A number of cell structures of the transmitting tissue in the stigma, style and the ovary papillar cells show no differences during development. The nucleus remains elongated and has some lobes, and no differences are observed in the karyoplasm. A nucleolus is present and has a compact constitution, and sometimes a granular part is observed. In the cytoplasm polysomes and monosomes are always present in a comparable quantity. Also the ground plasm shows no remarkable differences and contains fine granular and fibrillar material. The cells are connected by plasmodesmata, especially in longitudinal direction (*fig. 4*) Outside, against the cuticles electron dense material may be deposited (*fig. 5*). Cortical microtubules are observed in cells, in the lower part of the style.

The papillar cell wall and the inner epidermis cell wall at the top of the style consists of two distinct layers, a small one and a thick one covered with a undulating cuticle (*fig. 3*). In the inner epidermis cell walls in the lower part of the style, the part of the cell wall below the cuticle is somewhat irregular and viscous, showing fine electron dense threats (*fig. 5*).

In all stylar cells different patterns of vacuolation may be observed. The content of the vacuoles can be electron transparant or contain electron dense material. Sometimes there are vacuole-like inclusions. At the start of the open flower period in the papillar and subpapillar cells, these inclusions are electron dense. The same material can be also found in the cytoplasm (*fig. 4*). Very often the tonoplast is partly or totally absent during vacuolation. Around a zone without cell organelles short strands of endoplasmic reticulum (ER) are present, sometimes with a small vacuole originating from fusion of the ER-cisterns (*fig. 6*). A small number of desintegrated cells is sometimes observed at the base of the style as well as between the ovary papillar cell after pollination.

During the development there are also some distinct differences in the cellular contents of the stigmatic papillar, the subpapillar, the stylar transmitting and the ovary papillar cells. Just before anthesis, in all cells a number of vacuoles is present, most of the dictyosomes share vesicles and all plastids contain starch. The transmitting stylar middle and basal cells contain a few lipid granules, the

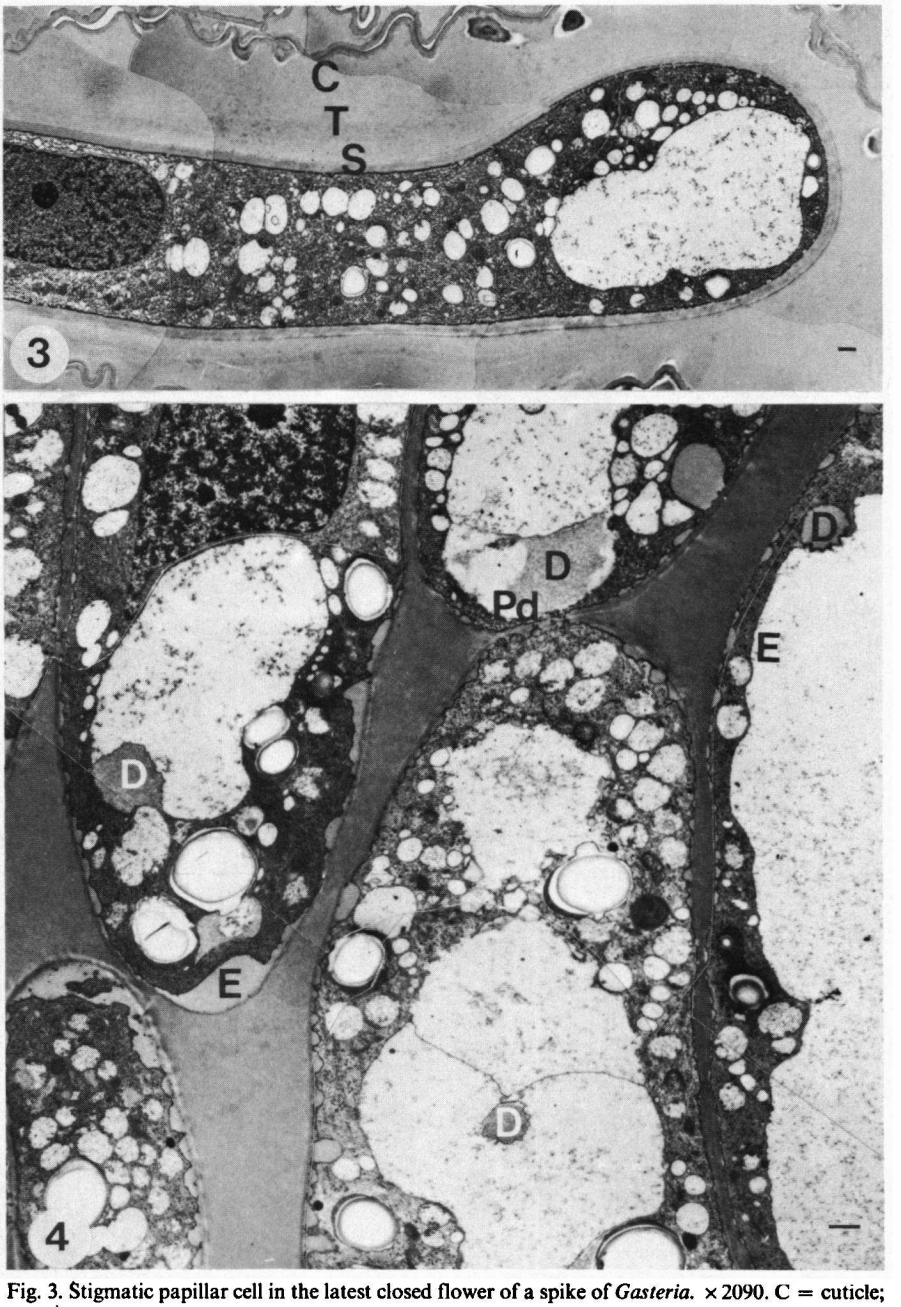
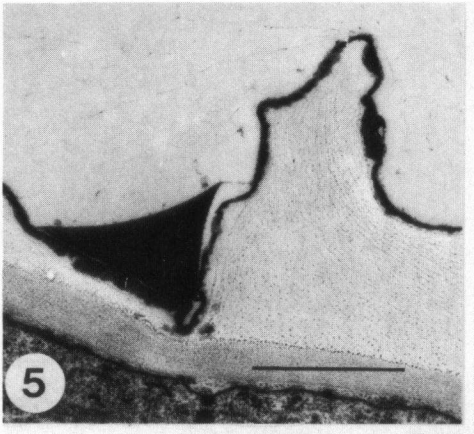


Fig. 3. Stigmatic papillar cell in the latest closed flower of a spike of *Gasteria*. ×2090. C = cuticle; T = thick cell wall layer; S = small cell wall layer. (Unless stated otherwise, the line on the figures represent a length of 1 μm.)

Fig. 4. Subpapillar cells of the latest closed flower of *Gasteria*. In the vacuoles and cytoplasma electron dense material is present (D). Some excretion occurs along the plasma membrane (E). Pd = plasmodesmata. ×3850.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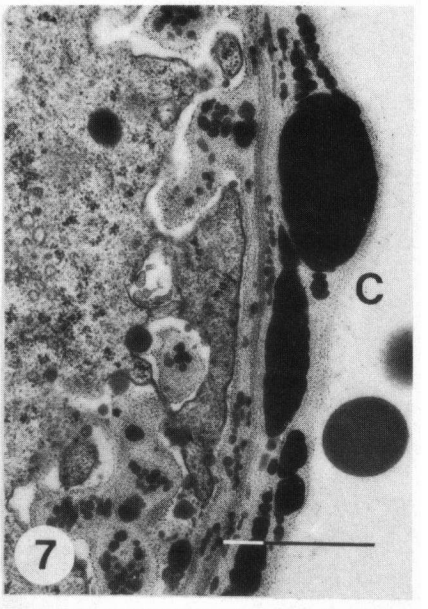
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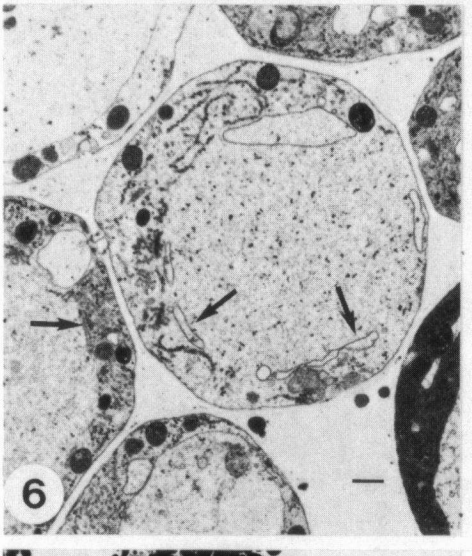
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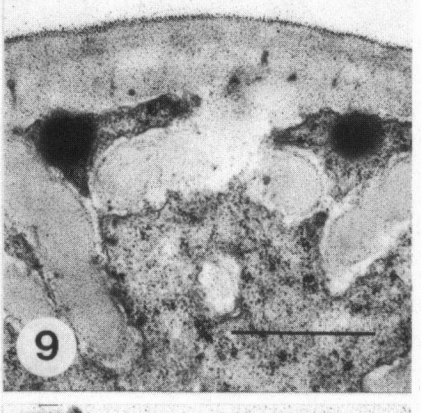
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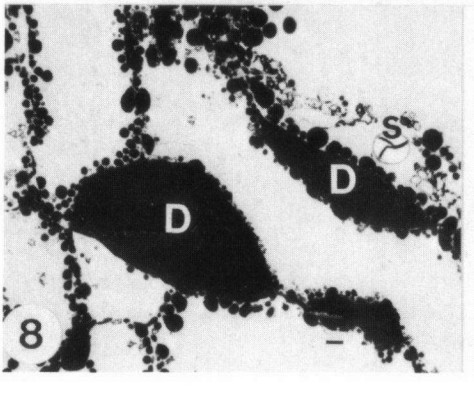
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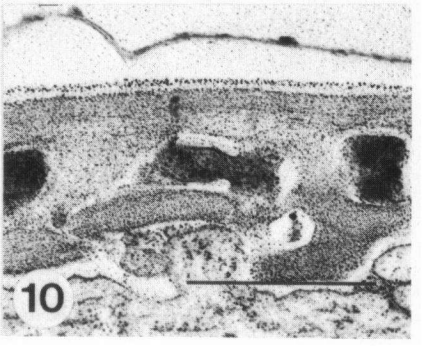
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stylar top cells contain more lipid. Mitochondria of the papillar and subpapillar cells and stylar top cells, contain less cristae compared with the mitochondria of the stylar middle and basal cells. The stigmatic papillar, the subpapillar and the ovary papillar cells show plasma membrane undulations. Elongated rough ER (RER) and smooth ER (SER) is observed in the stigmatic papillar cells and elongated RER in the transmitting tissue at the base of the style. In the cell wall of the transmitting tissue cells, osmiophylic granules become visible.

Between early and late stages of flower development, no changes are observed in the SER and the vesicles. Elongated RER appears in the transmitting cells at the middle and the base parts of the style. In these cells the lipid granules disappear. In some of the stigmatic papillar, subpapillar and connected stylar top cells changes occur in the content of lipid granules. In all cells a decrease in starch is observed in the plastids. From all cells an excretion of osmiophylic in the direction of the stylar canal takes place. This excretion is the most evident in the transmitting stylar middle cells. The osmiophylic granules both in a line outside the undulating plasma membrane and originate from the cytoplasm (*fig. 7*). Most granules are situated near remnants of cuticular fragments (*fig. 8*). In the subpapillar cells and the stylar top cells, vesicle-like and fine fibrillar structures are visible against the first wall layer. In the other transmitting cells lomasomes are observed. At the same developmental stage the ovary papillar cell wall has many undulations with lipid droplets in between them (*fig. 9*).

When the style is mature and the stigma has a small droplet of exudate the major cellular changes are observed. In all cells, lipid granules increase in number in contrast with the amount of vesicles and the dimension of the starch grains which decrease. Except in the stigmatic papillar and the ovary papillar cells, there is an increase in SER. No vesicles are excreted by the dictyosomes of the stigmatic papillar, subpapillar and the stylar top cells. At this stage elongated RER is only observed in the transmitting stylar middle cells and in the ovary

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Fig. 5. The cell wall along the canal of the stylar middle cell of a latest closed flower of *Gasteria*. Note the fibrillar structure in the cell wall layers. Outside the cuticle an electron dense deposit is present. × 20500.

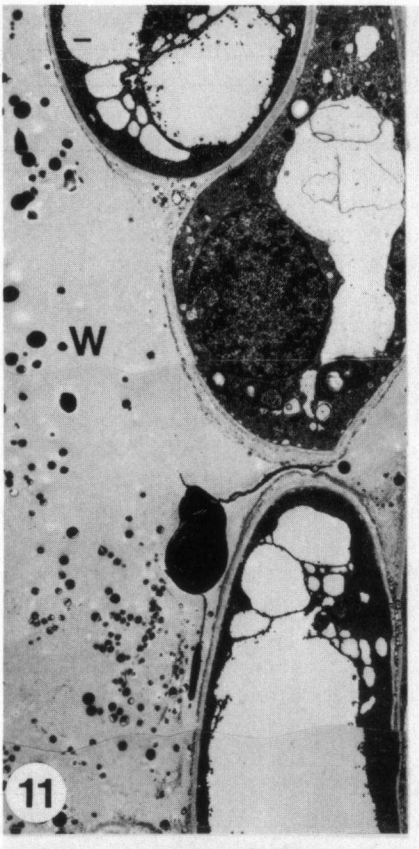
Fig. 6. Stylar middle cell along the canal showing the process of vacuolation in a mature style with exudate of *Gasteria*. Around the central part of the cytoplasm pieces of ER are present (arrow) which is observed in all stages. × 4500.

Fig. 7. Excretion of osmiophilic granules of a stylar middle cell along the canal of a mature style of *Gasteria*. C = canal. × 20500.

Fig. 8. Remnants of cuticles in the centre of the stylar canal of *Gasteria*, set with osmiophilic granules. Note the starch from a lysed cell (S). In between the cuticles electron dense deposits are visible (D). × 2130.

Fig. 9. Ovary papillar cell of the elongated style of *Gasteria*. In between the undulations lipid droplets are present. × 19850.

Fig. 10. Ovary papillar cell of a mature style of *Gasteria* with transfer wall after pollination. × 24950.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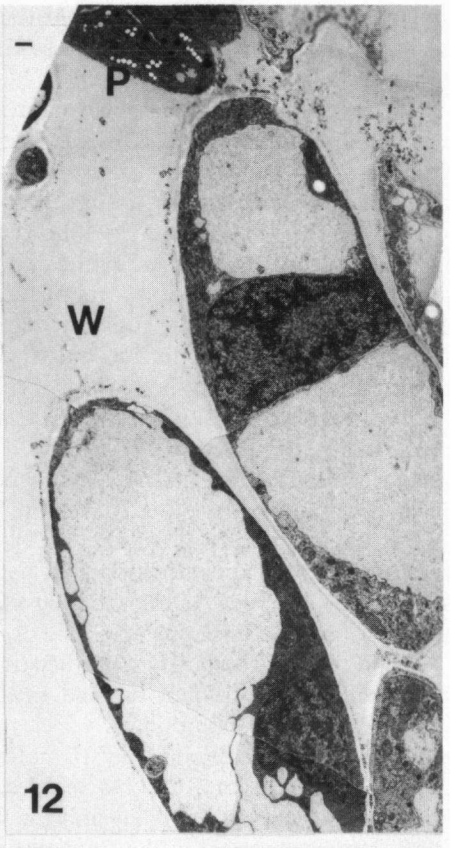
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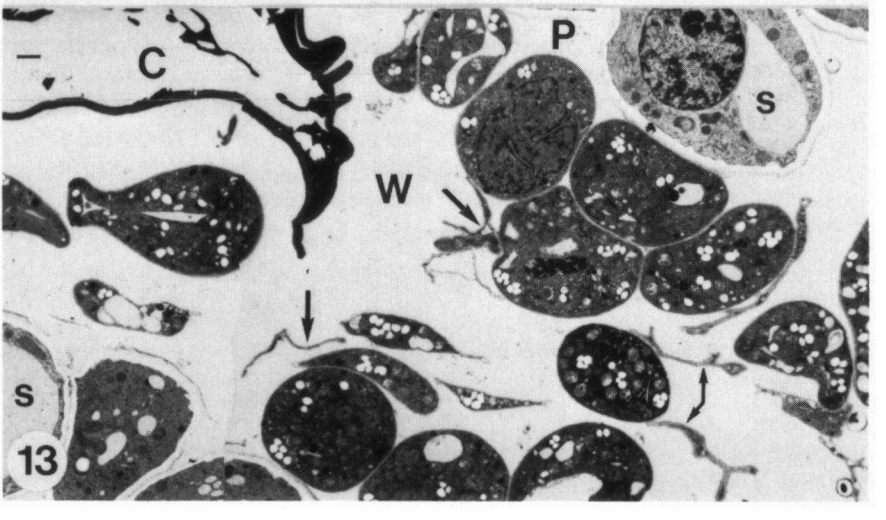
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papillar cells. The cell wall structure is comparable with the earlier stage, however, more osmiophylic granules are present, expecially against its first wall layer.

When pollen are germinated, only few changes occur in the first stylar transmitting cells. The SER cisterns in the stigmatic papillar cells dilate and at the zone of the pollen tube tip, the dictyosomes produce some vesicles. Another feature is the decrease in the number of mitochondrial cristae. Along the pathway of the pollen tube in the papillar, subpapillar and the stylar top cells, the number of vesicles and the amount of the osmiophylic granules in the wall decreases (*figs. 11, 12*). The ovary papillar cell gets more lipid droplets against its wall protrusions (*fig. 10*). The germinated pollen grow into the wall of the stigmatic papillar cell and from there into the solid wall material along the stylar canal. In this region many of the pollen tubes collaps (*fig. 13*). All these data are summarized in a scheme (*fig. 14*) and a survey (*fig. 15*).

3. DISCUSSION AND CONCLUSION

In the open flower of *Gasteria* major structural changes occur from the early stages of stylar development until the acceptance of the pollen and the subsequent pollen tube growth.

A change related to the development is the decrease of starch as a source for energy. The formation of the stylar canal content as nutrition for the pollen tubes is a continuous process. Till the receptive stage, two components are excreted: 1) an electron transparant material, probably formed by the dictyosomes especially in the lower part of the style and 2) a high amount of osmiophylic granules along the canal. Before the receptive stage, a decrease or an absence of lipid granules in the cytoplasm is observed, especially in the lower part of the style. Perhaps this decrease may be related to the formation of the osmiophylic granules.

The structure of the style with a very small canal at the top and a more liquid phase in the lower part can be explained as follows. In theory the viscous content of the stylar canal may be considered as a fluid substance, an exudate, but also as a less polymerized continuation of the solid cell walls of the transmitting tissue at the top of the style. In function the upper part of the style of a succulent as *Gasteria* is more solid and covered with a thick cuticle preventing evaporation.

Fig. 11. Osmiophylic granules in the subpapillar stylar wall material (W) of a mature style with exudate, unpollinated, of *Gasteria*. ×2150.

Fig. 12. Subpapillar stylar wall material (W) of a mature *Gasteria* style with exudate after pollen germination, osmiophilic granules are absent. P = pollen tube. ×2150.

Fig. 13. Cross section of the stylar top zone with the canal (C) and wall (W) material. Note the pollen tubes (P) growing in the wall of the stylar canal cells of *Gasteria* (C). Note the collapsed pollen tubes (see arrows). ×2550.

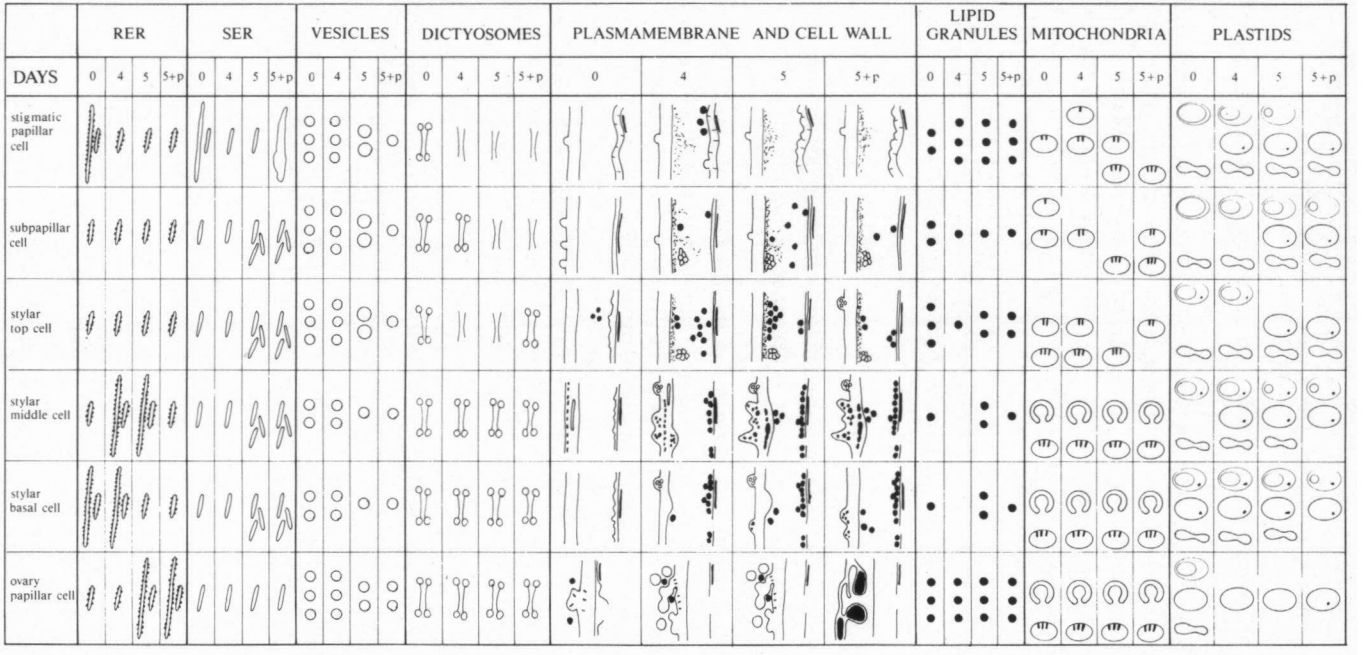

Fig. 14. Schematic presentation of cell organell analysis during stylar development in the open flower of *Gasteria*. Days 5 + p means pollinated stigma. The variation dimension of the thick cell wall layer is omitted. The black dot in the plastids represents the carotenoid, the open circle the amount of starch. From each stage at least three cells are analysed.

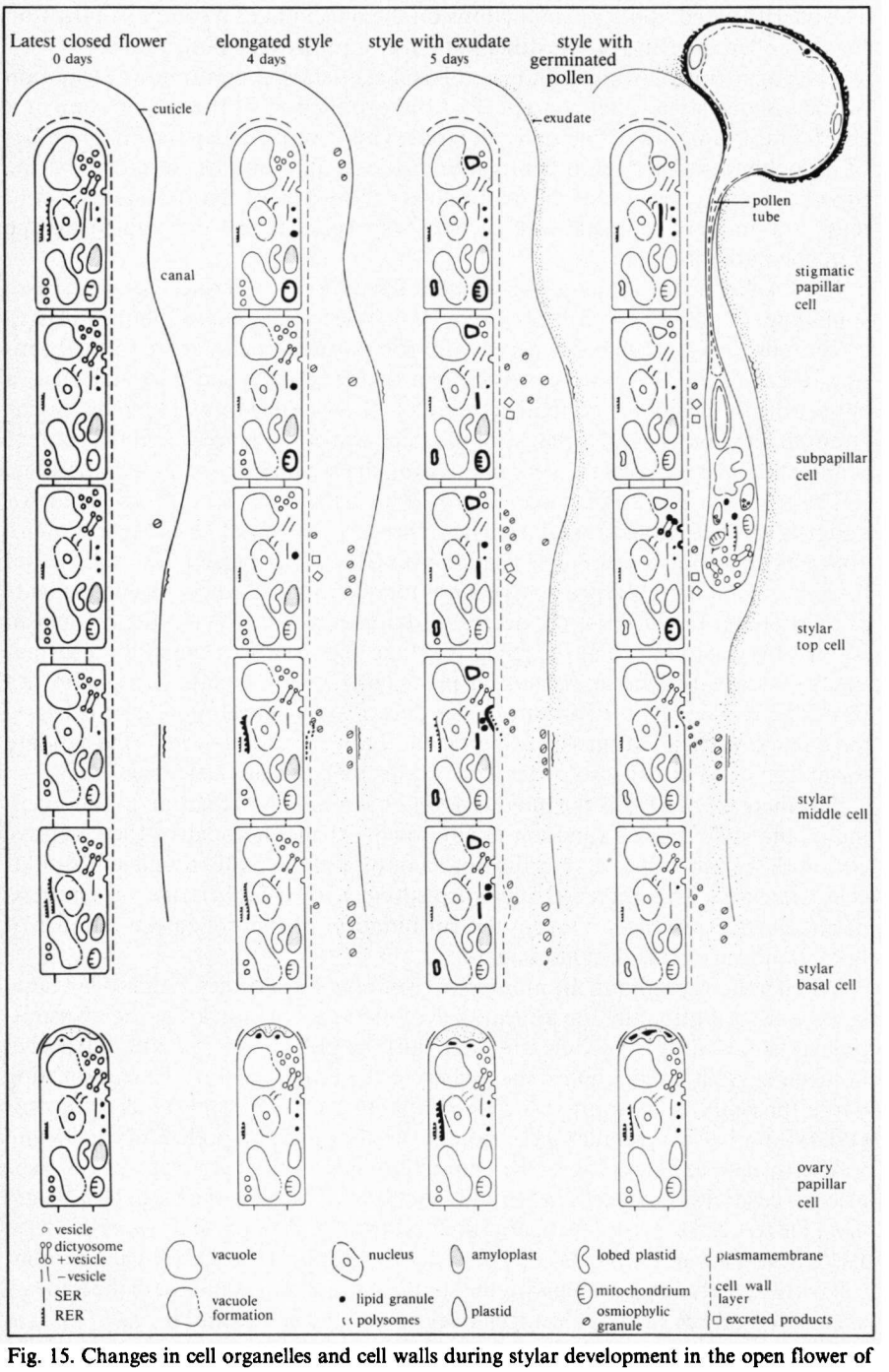

Fig. 15. Changes in cell organelles and cell walls during stylar development in the open flower of *Gasteria*. The heavy lined organelles represent the main changes in comparison with the previous stages.

The nearly closed upper part functions on the one hand as a source of nutrition for the pollen tube and on the other hand it compresses the pollen tube.

The stylar transmitting middle and basal cells show a continuous formation of the viscous style canal content. For the production of this stylar content a considerable amount of energy is required. This is reflected by the high number of mitochondrial cristae, in contrast with the smaller number of cristae at the top of the style. Because of the continuous production of the viscous style content, it ultimately protrudes out of the stylar canal at the top and forms the exudate on the stigma.

In all cells, different stages of vacuole formation are found. In some cases similar phenomena, as were reported by AMELUNXEN & HEINZE (1984) and HILLING & AMELUNXEN (1985), are observed. Probably new vacuoles are formed continuously and in all cells. A possible consequence of vacuole formation is a change in the vacuolar content as well as the osmolarity of the cells. Stylar strength remains assured, because of the thick walled upper part and the content of the canal and perhaps the very undulating thick cuticle surrounding the style.

The vacuolar content is also related to be a place for storage as precursor material for the stigmatic exudate. The stylar cortical cells of *Ornithogalum* (TILTON 1980) show also secondary vacuolation which may represent a mechanism for a particular metablic process, perhaps related to the synthesis of components of its exudate. In *Gasteria,* the decrease in number of vesicles in the cytoplasm fusing into vacuoles just at the receptive stage may be also a signal for a formation of a special vacuolar content as proteins (GAWLIK 1984). This fusion can be added to the vacuole formation. So during stylar development in *Gasteria* the change in vacuolation seems important. However, a relation to the exudate formation or its possible role in pollen tube growth can not be stated.

The increase of SER is the most general phenomenon in relation to the ripening of the style, but its function is unknown. At the moment of the receptive period the number of cristae in the mitochondria of the papillar and subpapillar cells increases. This increase may correspond with a higher rate of synthesis of energy-rich products. During the germination of the pollen the number of mitochondrial cristae decreases again.

When pollen germinate the number of osmiophylic granules in the stylar canal is reduced. Additionally the appearance of dictyosome vesicles in the transmitting stylar top cells is probably related with the pollen tube growth. Such phenomena suggest a reaction on the arrival of the pollen tube in the transmitting tissue, possibly in relation to its nutrition. In *Lilium* CAMPBELL & LINSKENS (1984) found also a production of exudate as a reaction on pollen tube growth.

The ovary papillar cell may be compared with a secretory transfer cell as is also the case with canal cells in lily and the so-called obturator cells in *Ornithogalum* (TILTON & HORNER 1983), and the nectaries of *Aloë* or *Gasteria* (HEINRICH 1975; SCHNEPFF & PROSS 1976). The cellular composition of the ovary papillar cell is only partly comparable with the stigma papillar cell, similar are the appearance of long RER strands along the nucleus and the use of starch.

On account of its morphology the style of *Gasteria* may be divided in a stylar

top part and a basal part. The top part contains a higher rate of lipid granules and vesicles, less production of dictyosome vesicles, a low number of mitochondrial cristae and an absence of osmiophylic granules in the thick cell wall layer, compared with the basal part. Less distinction can be made in the content and appearence of RER, SER and plastids. The wall lining the stylar canal, shows a gradient of osmiophylic granules and lower viscosity in basal direction. However, it cannot be excluded, that the osmiophylic granules are translocated to the upper part as a part of the excudate.

Along the whole pathway of the pollen tube osmiophylic material is present. Probably this material, together with the cellular remnants of the collapsed cells and carbohydrates is used for the growth of the pollen tube.

In function, the open style of *Gasteria* is to be regarded as a closed canal, a pathway for the pollen tubes. This canal consist of soft cell wall material for the nutrition of the pollen tube. These features are comparable with the intercellular growth, which occurs in the transmitting tissue of solid styles.

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