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MORPHOLOGICAL AND QUANTITATIVE CHANGES IN THE POPULATION OF CELL ORGANELLES DURING MICROSPOROGENESIS OF GASTERIA VERRUCOSA

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SUMMARY

The morphological changes in the nucleus and cell organelles of *Gasteria* during the development of the pollen mother cell into the ripe pollen are described and discussed. From the tetrad stage till after the first mitotic division the plastids contain electron transparent granules. The number of cristae in the mitochondria increases after the first mitotic division.

When the pollen wall is formed the excretion of the Golgi vesicles' content and the contact of the plasma membrane with the callose wall determine the template of the pollen wall pattern. The formation of the material for the bacula, followed by that of the tectum, and finally by that for the footlayer, takes place on the plasma membrane. The excretion of the fine fibrillar content of the Golgi vesicles proceeds till after the formation of the footlayer. The excreted material remains between the bacula.

The quantitative approach revealed that during meiosis the number of cell organelles remains constant. However, an increase in number takes place first after the pollen mother cell stage and before leptotene, secondly after the first mitotic division.

The data are compared with the previous results obtained with *Pinus sylvestris* (WILLEMSE 1971a, b, c).

1. INTRODUCTION

In previous studies on *Pinus sylvestris* different events during the microsporogenesis were described (WILLEMSE 1971a, 1971b, 1971c, 1971d). This study deals with the morphological and quantitative changes of cell organelles during the microsporogenesis of *Gasteria verrucosa*. The different developmental stages of this plant are easy to collect (STRAUB 1937). The results will be compared with the more extensive data obtained with *Pinus sylvestris*.

2. MATERIAL AND METHODS

2.1. Morphological investigations

At different stages of development of *Gasteria verrucosa* (Mill.) Haw. pieces of anthers were put in a buffered solution of 5% glutaraldehyde pH 7.2 at 0 °C for 22 hours. After washing in the same buffer solution, the specimens were put either in 1% buffered OsO_4 pH 7.2 at 0 °C for 45′ and subsequently stained in 1% uranyl acetate for 30′, or in a buffered solution of 1% $KMnO_4$ pH 7.2 for 45′. After dehydration in alcohol the specimens were embedded in Epon 812 and sectioned with a Porter-Blumm ultramicrotome. The sections prepared with

OsO_4 were poststained with lead citrate (REYNOLDS 1963). The material was examined in a Philips EM 300 electron microscope at 60 KV.

2.2. Quantitative approach

The following stages of microsporogenesis were selected: 1. pollen mother cell (PMC), 2. zygotene (Z), 3. prophase II or prometaphase II (P II), 4. early tetrad stage (ET), 5. young microspore (YM), 6. microspore in which the generative cell lies free before it starts to increase in length (M).

Countings were carried out on electron microscopical (EM) photographs of the sections of complete cells. Of one anther 20 different cells were counted. The cells were fixed in $KMnO_4$ and clear photographs of cell sections in the different stages of development were selected, while in every cell section a large part of the nucleus or two nuclei had to be present.

The area of the cytoplasm and the total cell area without the cell wall were determined in the same manner as described previously (WILLEMSE 1971c). The mean values and standard deviations of the number of plastids with granules, plastids, mitochondria, lipid granules, Golgi bodies and vesicles or small vacuoles per unit of cytoplasm ($5.9 \times 5.9\ \mu m^2 = 100$ points) were calculated from cell sections of EM photographs at a magnification of 7,500 $\times$.

2.3. Statistical analysis

The one way analysis of variance was applied to the mean value and standard deviation of the number of counted cell organelles per unit of area of cytoplasm. If the result was significant at the 5% level, it was investigated with SCHEFFÉ's (1959) or TUKEY's test for multiple comparison for each pair of mean values whether they are significantly different from each other, at a simultaneous significance level of 5%. TUKEY's test (SCHEFFÉ 1959), which is more powerful, can only be applied if the number of observations are equal, in other cases Scheffé's test has been applied. It is to be remarked that with Scheffé's test no such pair of mean values could be distinguished, altough the analysis of variance gives a significant result. The same test was applied to the data concerning the area of the nucleus, the cytoplasm, and the total area of the cell section.

3. RESULTS

3.1. Morphological observations

3.1.1. The pollen mother cell stage until diplotene

The pollen mother cell has a large nucleus with a heterogeneous nucleolus. The plastids contain membranes. The mitochondria have few cristae and a spherical or rod-like shape. The Golgi bodies produce only a few very small vesicles. Vesicles with an electron transparent content are present (*fig. 1*). Some strands of rough endoplasmic reticulum (RER) are dispersed throughout the cyto-

plasm. Many ribosomes and polysomes are present. The cell wall has plasmodesmata, during leptotene the cell wall is osmiophilic and thin.

During leptotene some lipid granules lie together and are connected with electron transparent vesicles and the whole group is surrounded by dark dots. This "lipid complex" is only visible after fixation with OsO_4 (*fig. 2*).

During zygotene and pachytene the synaptinemal complex is present in the nucleus (*fig. 3*). The nuclear pore is complex and has an annulus (*fig. 4*). In some places the presence of short extensions is suggested, as was described in *Pinus* (WILLEMSE 1971a). There are only few changes in the cell organelles: now the Golgi bodies produce more vesicles and mainly ribosomes are present. The callose wall formation starts. Around the cell and against a thin layer of electron dense material the electron transparent callose wall appears. The cellulose wall between the cells remains still visible. The electron dense layer represents the thin new cell wall which is formed around each cell before meiosis starts. In these cell walls channels are observed through which organelles or a part of the nucleus may pass (*fig. 5*).

3.1.2. Diplotene until the tetrad stage

During diplotene the nucleolus begins to disappear, in the karyoplasm many granules appear. In the cytoplasm the volume of the plastids increases. Few lipid granules are now present. The Golgi bodies produce very small vesicles and concentric membranes are also observed (*fig. 6, 7*). With $KMnO_4$ fixation the ER appears locally against the plasma membrane (*fig. 7*). Microtubules are found near the plasma membrane mainly oriented in one direction. The thin new cell wall can be observed around the thick callose wall in which also gaps are visible, representing the places where channels were present before (*fig. 6*).

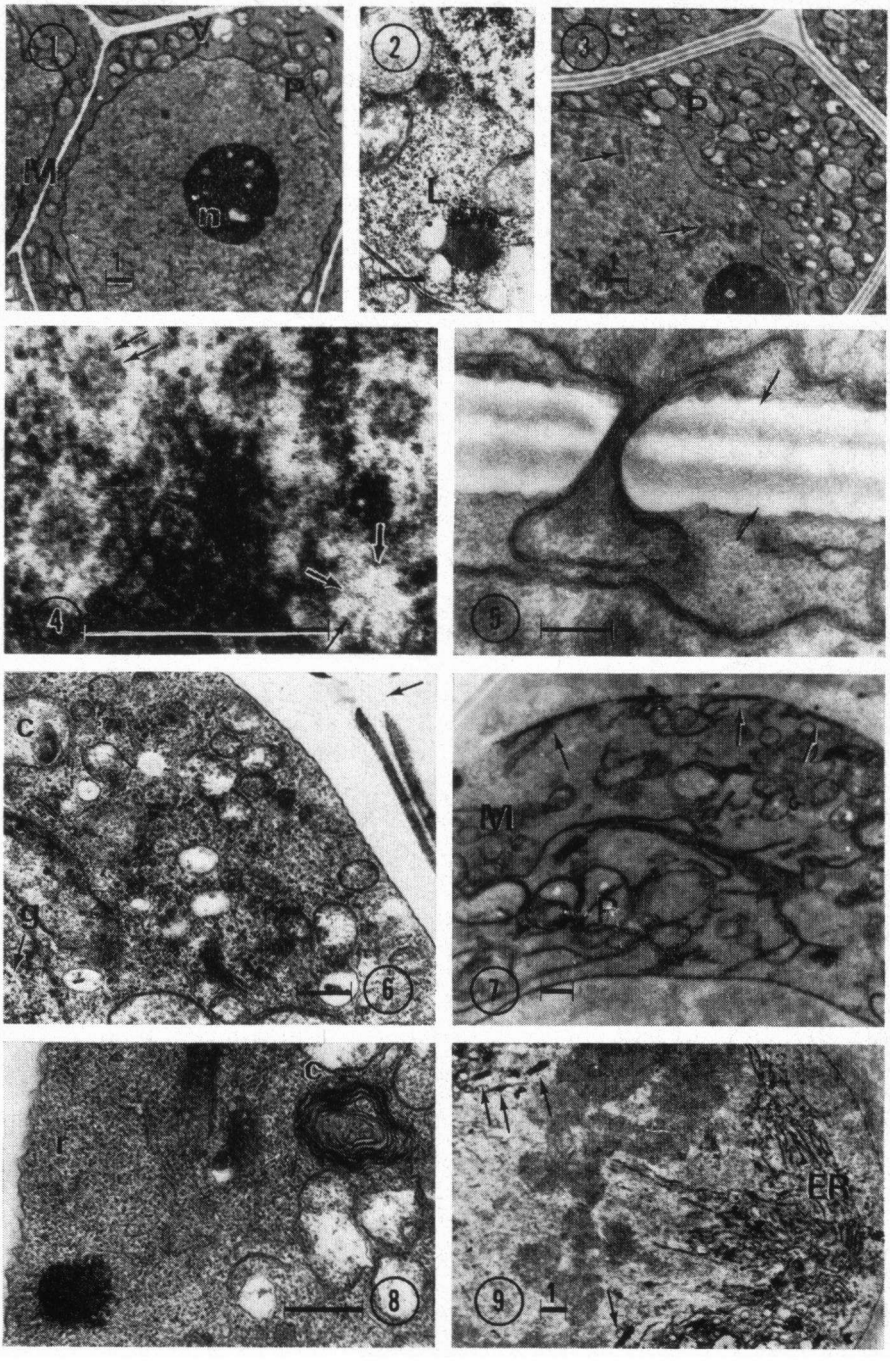
The tapetal cells show some orbicules outside the plasma membrane on which also electron dense granules are situated.

During the meiotic divisions the nuclear membrane is partly broken down. After the telophase the granules of the karyoplasm are mixed with the cytoplasm in which many ribosomes become visible. Especially when the first meiotic division takes place many vesicles are present. A centriole or polar body could not be observed. Remarkable is the presence of the ER strands in the polar region (*fig. 9*). In the cytoplasm nucleolar-like bodies are frequently observed; concentric membranes (*fig. 8*) remain present. During the divisions the Golgi bodies are situated near the nucleus or nuclear region (*fig. 9, 10*). There may be a relation between the presence of Golgi bodies which produce small vesicles and the partly rebuilding of the nuclear membrane (*fig. 11*).

During the short interphase II and prophase II the cell organelles lie between the two nuclei, whereas the mitochondria are mainly situated in the centre (*fig. 10*).

3.1.3. The tetrad stage

After the separation of the four microspores by a callose wall the chromatine in the nucleus remains somewhat contracted for a while. Invaginations of the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nuclear membrane are not observed. The nucleolar-like bodies remain visibl in the cytoplasm (*fig. 12*).

In some plastids electron transparent granules appear, probably as reserve food. Many lipid granules are present when the pollen wall formation starts (*fig. 12*). Mainly ribosomes are observed, some microtubuli and concentric membranes remain present. The callose wall is still growing around the microspore until the excretion of the Golgi material takes place. After poststaining electron dense spots become visible in the callose wall, probably on the border between the callose wall which was present before the tetrad stage and the one formed during the early tetrad stage (*fig. 23*).

3.1.4. Pollen wall formation

During the early tetrad stage the plasma membrane lies against the straight callose wall (*fig. 14*). In the cytoplasm the Golgi bodies start to produce electron transparent vesicles of different shape in which first an electron dense granule, thereafter fine fibrillar material is present (*fig. 13*). The content of the Golgi vesicles is excreted and appears between the plasma membrane and the callose wall. This excretion causes the undulations of the plasma membrane (*fig. 15, 16*). The contact between the plasma membrane and the callose wall persists locally for a while. In these places on the plasma membrane slightly electron dense material appears whereas the direct contact with the callose wall is severed. In this way the bacula appear (*fig. 17*). The excretion of the content of the Golgi vesicles continues and the plasma membrane retires further from the callose wall. The places where the plasma membrane makes contact with the bacula extend and the bacula become cone-shaped. The plasma membrane shows no undulations (*fig. 18*). Electron dense material may be observed now on the place of the tectum. The structure of bacula and tectum becomes well recognizable; their electron density seems to increase (*fig. 19*). The contact on the base of the bacula with the plasma membrane becomes less intensive (*fig. 20*). Now the footlayer is formed by the outgrowth of the base of the bacula. The plasma membrane is no longer in contact with the bacula (*fig. 21, 22*).

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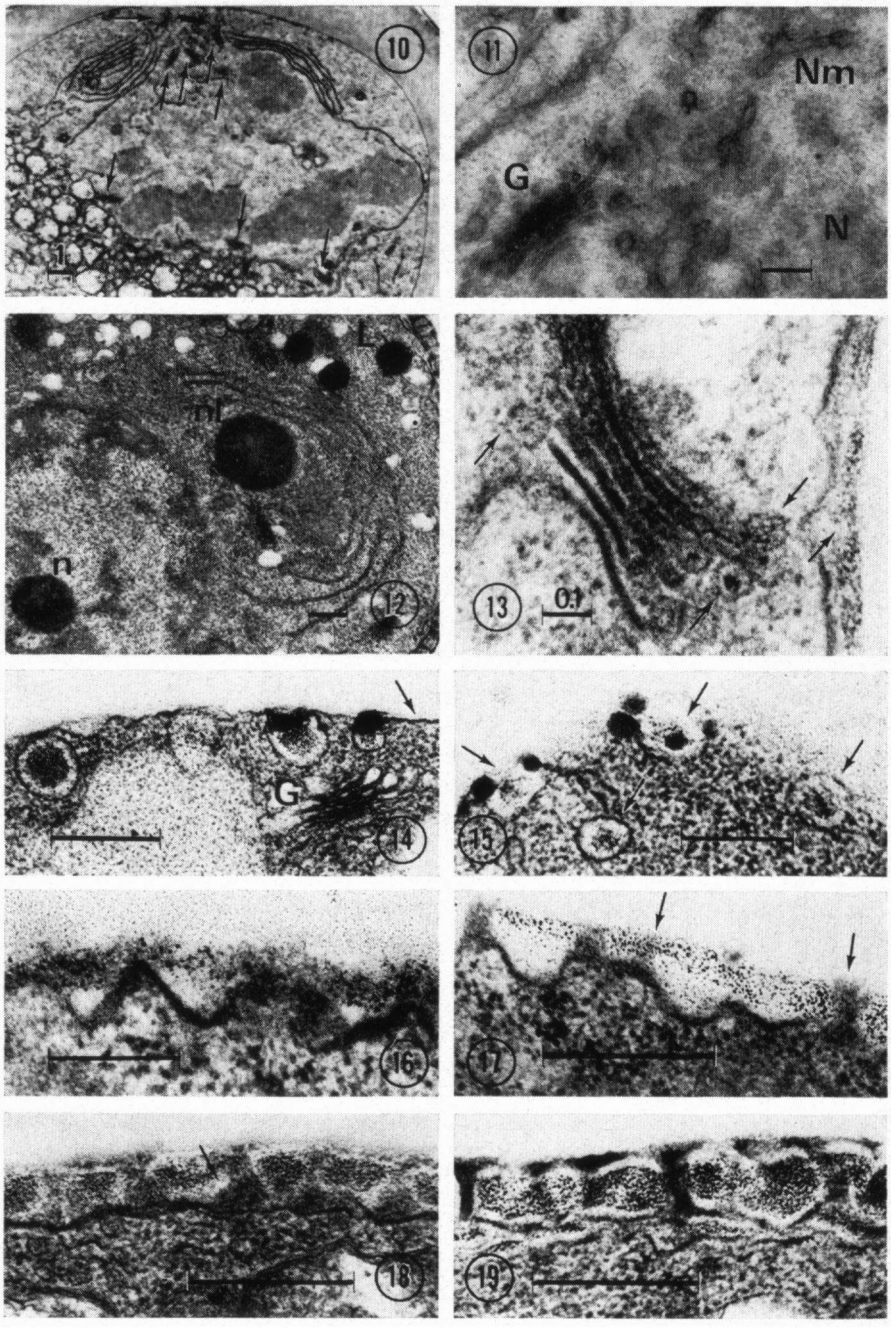
Fig. 1. Pollen mother cell with heterogeneous nucleolus (n), plastids (P), mitochondria (M) and vesicles (V), Glutaraldehyde (GA)-$KMnO_4$ fixation, × 3,680.
Fig. 2. Detail cytoplasm leptotene with "lipid complex" (L), × 13,000.
Fig. 3. Zygotene. Note the synaptinemal complex (arrows), GA-$KMnO_4$ fixation, × 4,000.
Fig. 4. Nuclear pore with annulus and the short extensions (arrows), × 77,000.
Fig. 5. Zygotene: cell wall channel with passing part of the nucleus. Note the callose wall (arrow), GA-$KMnO_4$ fixation, × 22,600.
Fig. 6. Diplotene: granules (g) in the karyoplasm. Concentric membrane (c) and remnant of a channel between two cells (arrow), × 13,000.
Fig. 7. Diplotene: ER along the plasma membrane (arrows), plastids (P) and mitochondria (M), GA-$KMnO_4$ fixation, × 8,300.
Fig. 8. Telophase II: many ribosomes (r) and a concentric membrane (c), × 23,000.
Fig. 9. Early anaphase I in the polar region ER. Golgi bodies around the nuclear region (arrows), GA-$KMnO_4$ fixation, × 3,600.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Just before break-out of the microspore, the electron density in tectum, bacula and footlayer increases. Between the plasma membrane and the footlayer and parallel to the plasma membrane thin membranes appear (*fig. 23*). After break-out of the microspore tectum, bacula and footlayer are not yet thickening. Probably the excretion of the Golgi material still takes place since Golgi vesicles are present in the cytoplasm. Besides, material originating from Golgi vesicles lies between the plasma membrane and the footlayer (*fig. 24*).

Around the young microspore the pollen wall thickens (*fig. 25*). The material originating from the Golgi vesicles remains present between the bacula of the microspore wall. Against the layer of Golgi material the pollen glue is visible on the pollen wall (*fig. 26*). Lamellae of unit membrane dimension are only observed in the footlayer against the plasma membrane where the pollen wall sculpture is absent near the colpus area (*fig. 27*).

In the cytoplasm the excretion of the content of the Golgi vesicles is blocked by a sheet of ER along the plasma membrane in the colpus area. This sheet seems to be in contact with the nuclear membrane (*fig. 28*).

During the tetrad stage orbicules and electron dense granules are observed along the plasma membrane outside the tapetal cell.

3.1.5. The microspore before and after the first mitosis

The young microspore increases in volume and gets some vacuoles and vesicles in the cytoplasm. All plastids contain electron transparent granules (*fig. 29*). Golgi bodies produce many vesicles. Packets of RER are formed in the cell principally near the nuclear membrane (*fig. 30*). Ribosomes as well as polysomes are present. The thin new cell wall remains intact after break-down of the callose wall and is visible against the tectum of the pollen wall for a long time. Before mitosis the formation of the intine starts in the area of the colpus (*fig. 29*). In this intine many thin extensions of the plasma membrane make contact with the

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Fig. 10. Prometaphase II: Golgi bodies around the nuclear region (arrows), GA-$KMnO_4$ fixation, × 2,900.

Fig. 11. Telophase II: Golgi body (G) with vesicles near to the rebuilding nuclear membrane (Nm). N: nucleus, GA-$KMnO_4$ fixation, × 13,000.

Fig. 12. Tetrad: compare the nucleolar-like body (nl) with the nucleolus (n). L: lipid granules, × 9,000.

Fig. 13. Golgi body during pollen wall formation. Note the content of the Golgi vesicles and the material outside the plasma membrane (arrows), × 69,000.

Fig. 14. Start of pollen wall formation: Golgi body (G) and plasma membrane against the callose wall (arrow), × 32,000.

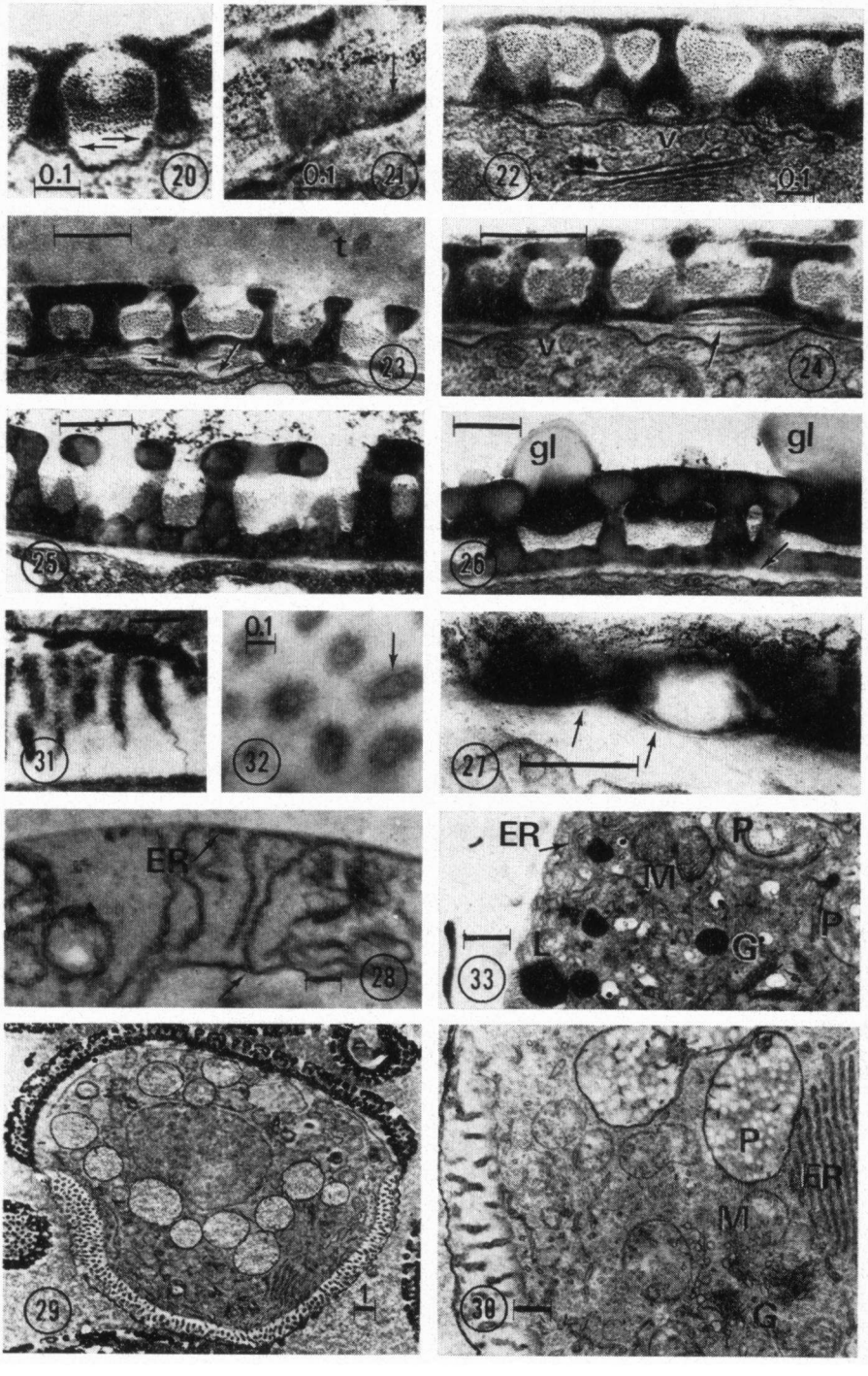
Fig. 15. Excretion of the content of Golgi vesicles (arrows), × 33,000.

Fig. 16. Undulating plasma membrane, × 40,000.

Fig. 17. Appearance of the bacula (arrows), × 51,000.

Fig. 18. Cone-shaped baculum (arrow), no undulating plasma membrane. Note the appearance of the tectum, × 47,000.

Fig. 19. Tectum and bacula get more contrast between the bacula material of the Golgi vesicles, × 47,000.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surrounding fluid. The extensions are only covered by a thin layer of an unsculptured exine (*fig. 31, 32*).

After mitosis, when the generative cell is formed, the cell organelles increase in number. In the plastids the electron transparent granules begin to disappear. The number of cristae in the mitochondria increases. The Golgi bodies produce only a few small vesicles. Many lipid granules appear. Among the organelles are ribosomes and short strands of ER (*fig. 33*). In the ripe pollen small vesicles become visible again (*fig. 33*).

In the plastids of the tapetal cell electron dense globules are formed which come out into the fluid around the microspores during breakdown of the tapetal cell.

3.2. Quantitative apoprach

Diagram 1 shows the changes in the cell section area, in the cytoplasm area, and in the nuclear area of the cell section during some different stages of microsporogenesis. The mean values with standard deviations of the number of plastids, mitochondria, lipid granules, Golgi vesicles, and vesicles (or vacuoles) are noted per unit of area of the cytoplasm (100 points). In the diagram the results of the Tukey's test are also included.

If the one way analysis of variance and the Tukey's test are applied to the number of cell organelles and to the cell section, cytoplasm, and nuclear area, significant results are obtained.

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Fig. 20. Contact between bacula and plasma membrane severs (arrows), × 57,000.
Fig. 21. Footlayer becomes visible (arrow), × 72,000.
Fig. 22. Formation of the footlayer by junction of the base of the bacula. Note the Golgi vesicles (v), × 40,000.
Fig. 23. Before break-out of the microspore. Membranes appear along the plasma membrane and the footlayer (arrows). Note the electron dense spots (t) in the callose wall, × 24,000.
Fig. 24. Pollen wall after break-out. Note the membranes (arrow) and Golgi vesicles (v), × 33,000.
Fig. 25. Pollen wall of the young microspore, × 20,000.
Fig. 26. Pollen wall of the ripe microspore with intine (arrow) and pollen glue (gl), × 21,900.
Fig. 27. Lamellae of unit membrane dimension (arrows), × 34,500.
Fig. 28. Early tetrad stage: sheet of ER along the plasma membrane (arrow). Note the contact with the nuclear membrane (arrow), GA-$KMnO_4$ fixation, × 11,000.
Fig. 29. Young microspore. Note the intine, GA-$KMnO_4$ fixation, × 2,800.
Fig. 30. Detail cytoplasm young microspore, plastids with electron transparent granules (P), mitochondria (M), Golgi bodies (G) and packet of ER, GA-$KMnO_4$ fixation, × 9,700.
Fig. 31. Extensions of the cytoplasm into the intine, GA-$KMnO_4$ fixation, × 12,500.
Fig. 32. Cross section of intine. Note the plasma membrane (arrow), GA-$KMnO_4$ fixation, × 36,000.
Fig. 33. Cytoplasm of the ripe pollen. Mitochondria with many cristae (M), plastids without electron transparent granules (P), lipid granules (L), Golgi bodies (G) and ER, × 14,800.
Unless mentioned otherwise, the line on the figures represents a length of 0.5 μm, and OsO_4 fixation was used.

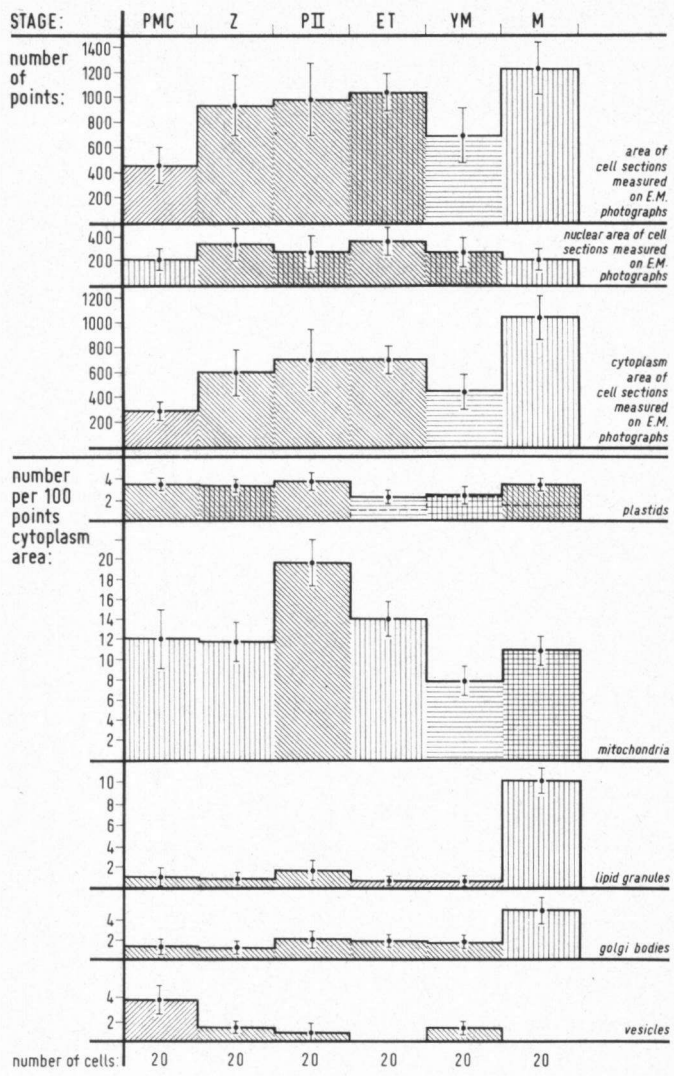

Diagram 1. The mean values and standard deviations of the cell section area, cytoplasm, and nuclear area of the cell section are given in number of points per stage of microsporogenesis as well as the number of organelles per 100 points cytoplasm area at the same stages. The dotted line represents the number of plastids in which electron transparent granules are present.

The results of the Tukey's test are indicated by means of an oriented shading. In columns with a corresponding type of shading the mean values do not differ significantly. A shading in two directions indicates that the mean value of this column does not differ significantly from all mean values of the columns with shading in each of the two directions of the double shaded column.

The number of cells counted is given on the base of the diagram.

4. DISCUSSION AND CONCLUSION

The results obtained with *Gasteria* show differences as well as similarities with respect to the microsporogenesis described for other plants and in *Pinus sylvestris* in particular.

4.1. Morphological changes in the nucleus

During zygotene and pachytene the synaptinemal complex is observed. During diplotene an increase of granules in the karyoplasm starts which is followed by a mixing of this material with the cytoplasm during the telophase. In telophase many ribosomes are present in the cytoplasm. Both phenomena are also observed in other plants including *Pinus*.

As in *Lilium* (DICKINSON & HESLOP-HARRISON 1970) in telophase I nucleolar bodies become visible in the cytoplasm. In *Gasteria* the nucleolar-like bodies are found from telophase up to the young microspore stage. In *Pinus* they are present from diplotene to interphase II. In general the nucleolar-like bodies may function as carrier of nuclear information to the cytoplasm; this may occur partly instead of the formation of invaginations in the nuclear membrane as in *Podocarpus* (ALDRICH & VASIL 1970) or in *Pinus* (DICKINSON & BELL 1970; WILLEMSE 1971b).

4.2. Morphological changes in the cytoplasm

In plastids of *Gasteria* reserve material appears as electron transparent granules from the tetrad stage until after the first mitosis of the microspore. The morphology as well as the time of appearance show no similarities with the presence of reserve material in the plastids of *Pinus* or *Tradescantia* (MARUYAMA 1968).

The cristae of the mitochondria increase in number after the first mitosis. This has been observed also in *Pinus sylvestris* (WILLEMSE 1971e) and in other plants (HOEFERT 1969; DEXHEIMER 1970).

In *Gasteria* a "lipid complex" is only present in the leptotene and zygotene, no further aggregation of lipid granules occurs such as has been observed in *Lilium* (HESLOP-HARRISON & DICKINSON 1967) or *Pinus*.

The Golgi bodies are always present. Production of vesicles takes place during zygotene to form the callose wall; during the division stages to rebuild the nuclear membrane; during the tetrad stage to form the pollen wall; and during the young microspore stage to form the intine. In *Pinus* the same relations and functions could be noted. Whether one generation of Golgi bodies supplies all these functions or more generations of Golgi bodies exist, as has been suggested in *Tradescantia* (MARUYAMA 1968), could not be demonstrated in *Gasteria*.

Vesicles appear in the pollen mother cell stage, zygotene, and after the breakout of the microspores. During the cell divisions small vesicles are present in the cytoplasm.

Packets of ER are observed only during the young microspore stage. Strands of ER are found in every stage of development. Concentric membranes are observed from diplotene to the young microspore stage; as may be partly the

case in *Pinus*, in *Gasteria* no relation could be established to the formation of new Golgi bodies as in *Tradescantia* (MARUYAMA 1968).

As in *Pinus*, ribosomes are always present in the cytoplasm of *Gasteria*. Polysomes are found in the pollen mother cell, during telophase with interphase II or early tetrad stage, and in the young microspore.

About the microtubules less information has been obtained.

As has been found in many other microspores, during the zygotene an intensive contact between the cells exists by means of channels (HESLOP-HARRISON 1971). In *Gasteria* the callose wall appears during the zygotene, in *Pinus* during the diplotene. The thin new cell wall around the microspore is also observed around the young microspores and seems to be resistant to the enzymes which break down the callose wall.

4.3. The pollen wall formation

In *Gasteria* the template of the pollen wall is formed by a local excretion of the content of Golgi vesicles and a brief contact between the plasma membrane and the callose wall. In the places where the contact between plasma membrane and callose wall persists, the production of material for the pollen wall starts immediately. First the bacula and thereafter the tectum and footlayer appear, still in contact with the plasma membrane. The area of the plasma membrane producing the material for the pollen wall increases during the pollen wall formation. As in *Pinus*, in *Gasteria* the template of the pollen wall pattern also depends on the local excretion of the content of Golgi vesicles and on the contact between the plasma membrane and the callose wall.

The material for the pollen wall originates probably from the cytoplasm of the microspore. In *Gasteria*, as in *Pinus*, many lipid granules are present during the pollen wall formation. The production of the material for the pollen wall takes place on the plasma membrane. Lamellae of unit membrane dimension are only observed in the basal layers of the footlayer near the intine of the colpus. Remarkable is the increase in electron density of the bacula and tectum during the formation of the pollen wall. The suggestion can be made that the pollen wall consists of two kinds of material: less electron dense material and electron dense material, which may be the sporopollenin. The other possibility is that the sporopollenin is preceded by a less electron dense precursor. The material for the pollen wall may be transported around the excreted Golgi material and may probably in this way reach the tectum and partly the footlayer.

Compared with the pollen wall formation in *Pinus sylvestris* the one in *Gasteria* shows some differences. In *Pinus* more of the material of the Golgi vesicles is excreted, however, for a shorter duration. This material is included in the pollen wall and precipitates against the protrusions of the callose wall which are formed on the plasma membrane. The callose wall formation in *Pinus* proceeds for a long time. Thereby the production of material for the pollen wall takes place on membranes partly inside and partly outside the cytoplasm. In *Gasteria* fewer Golgi vesicles are produced and the excreted material is probably only for a very small part included in the bacula, tectum, and foot-

layer. The production of callose on the plasma membrane stops when the excretion of the content of the Golgi vesicles starts.

From these results it appears that the pollen wall pattern and formation may depend on the quantity of excreted Golgi material, the duration of the callose wall formation, the start of the production of pollen wall material on the plasma membrane, and finally on the participation of the excreted Golgi vesicle material in the formation of the pollen wall.

In *Pinus* the material of the Golgi vesicles is used both as material for the pollen wall and to push off the plasma membrane. In *Gasteria* this material which remains in the pollen wall as a distinct layer between the bacula seems especially necessary for pushing off the plasma membrane and blocking further penetration of the pollen glue. The material of the content of the Golgi vesicles probably consists of a polysaccharide and is less resistant than cellulose (WILLEMSE 1972). In both pollen types the excretion of the content of Golgi vesicles determines the pattern of the pollen wall.

The participation of Golgi material in the formation of the pollen wall pattern may be deduced from the undulations of the plasma membrane, the presence of the content of Golgi vesicles outside the plasma membrane, and the presence of a sheet of ER in the area of the colpus which blocks the formation of a sculptured pollen wall because of the arrest of the Golgi vesicles. Whether the transport of Golgi vesicles is active or passive is not clear.

In *Pinus* and *Gasteria* the pollen wall thickens outside the callose wall during the young microspore stage. During the pollen wall formation orbicules and electron dense granules are present outside on the plasma membrane of the tapetal cell. The electron dense granules produced in the plastids of *Gasteria* may be a pigment or a pigment precursor for the anthocyanins (WIERMANN & WEINERT 1969).

4.4. The quantitative approach

Although a greater number of cells was counted per stage and fewer but more distinct stages could be used, all problems around the quantitative approach remain the same as discussed previously (WILLEMSE 1971c).

The area of the cell section and the cytoplasm area increase: first from the pollen mother cell stage up to the tetrad stage and secondly from the young to the ripe microspore stage. The area of the nucleus shows some fluctuations. The area of the microspore is the largest. During meiosis the area of the cytoplasm and of the total cell increases both in *Gasteria* and in *Pinus*.

The density of cell organelles per unit of cytoplasm area of the pollen mother cell stage and the zygotene does not differ significantly except for the vesicles or the vacuoles. This means that plastids, mitochondria, lipid granules and Golgi bodies increase about twice in number with the increase of the area of the cytoplasm. A greater increase in number of the same organelles occurs in the microspore stage: compared with the young microspore stage the density of organelles and the area of the cytoplasm increase. Compared with the zygotene or the early tetrad stage the increase in number is less excessive for the plastids

and mitochondria. The increase in number of cell organelles after the first mitosis has been reported in *Pinus sylvestris* (WILLEMSE 1971e) and in many other plants (SANGER & JACKSON 1971; VAZART 1971).

During the prophase II the density of cell organelles, except for the vesicles, is high. This phenomenon is due to the localization of all organelles between the two nuclei. The mitochondria are all located in the centre of the cell, whereas the plastids, vesicles and lipid granules are situated in the vicinity of the cell centre but between the two nuclei. Golgi bodies lie around the nuclei. Because of the presence of two nuclei in the selected cell sections a higher density of cell organelles per unit of cytoplasm may be expected. Besides, no cell organelles disappear during the following stages.

Except for the vesicles, no change in number of organelles occurs between the zygotene and the early tetrad stage. The low number of plastids in the early tetrad stage is a border-line case (compare the mean values of the early tetrad stage and young microspore stage with the zygotene). This means that during meiosis no increase in the number of plastids, mitochondria, Golgi bodies, and lipid granules occurs. The same has been demonstrated in *Pinus*.

As could be expected, the decrease of the cytoplasm area during the young microspore stage and the constancy or decrease of the density of cell organelles per unit of cytoplasm reveal that the number of organelles has decreased in the young microspore cell, compared with the preceding stages.

The density per unit of cytoplasm and number of vesicles or vacuoles during the microsporogenesis show great fluctuations.

The average number of organelles per unit of cytoplasm area in *Gasteria* resp. *Pinus* shows some similarities in the number of plastids and Golgi bodies. However, in *Pinus* fewer mitochondria but more lipid granules are present than in *Gasteria*.

Although in phylogenetical sense the pollen of *Gasteria* (Angiospermae, Monocotyledoneae) should be more highly developed than that of *Pinus* (Gymnospermae), the distinctness on a morphological level during microsporogenesis appears to be very small.

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