AN ABSTRACT OF THE THESIS OF

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in BOTANY (Major) presented on 7/9/70 (Date)

Title: LIGHT AND ELECTRON MICROSCOPY OF CHLOROPLASTS IN EQUISETUM GAMETOPHYTES

Abstract approved: Redacted for Privacy

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Spores of Equisetum were germinated and the resulting gametophytes grown to maturity in standard microcultures. Light and electron microscope observations were made of the chloroplasts within the gametophytes.

These chloroplasts were found to be oval to dumb-bell shaped organelles possessing organized grana. By means of time-lapse movies they were shown to replicate by fission within the meristematic cells of the thalli. Plastid divisions were most often found in cells which had just divided. Plastid division required from eight to 30 hours. No proplastids were seen in the developing gametophytes. The plastids became degenerate in the rhizoids and in the developing sperm.

Light and Electron Microscopy of Chloroplasts in Equisetum Gametophytes

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1971

APPROVED:

Redacted for Privacy

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Date thesis is presented 7/9/70

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INTRODUCTION

Replication and ultrastructure of the chloroplasts of *Equisetum* gametophytes have not been reported previously. Lack of information for *Equisetum* may be due, in part, to the difficulties encountered in finding or growing *Equisetum* gametophytes throughout the year. Usually spores of *Equisetum* remain viable for only a few days under room conditions and so are available for culturing only a short while each year. Once we had overcome the problem of spore storage we found the cultured gametophytes to be excellent experimental materials for studies of chloroplast development.

Equisetum gametophytes are often small enough when mature that the entire thallus can be easily grown within a microculture chamber on a standard microscope slide. The gametophytes consist of filaments and lobes of cells thin enough to allow direct microscopic examination of chloroplasts within individual living cells.

A preliminary study of cultured *Equisetum* gametophytes indicated that mature chloroplasts within them did divide. Therefore, a study of the details of *Equisetum* chloroplast replication, using both light and electron microscopy, was initiated.

REVIEW OF LITERATURE

That chloroplasts replicate was first suspected in 1846 when Naegeli noted that there was a 50% increase in plastid number from one internodal area to the next in the alga Nitella. In 1882 plastid division was observed in Spirogyra by Strasburger (Kirk and Tilney-Bassett, 1967). Meyer, in 1883 (Kirk and Tilney-Bassett, 1967), and Schimper (1883) described chloroplasts as individual organelles which divide much as a cell divides and are continuous from one generation to the next. Their theory -- that all plastids are derived from pre-existing plastids, possess many autonomous characteristics, and are transmitted from cell to cell during vegetative and sexual reproduction -- has been supported by practically all observations made since it was put forth.

Reports of chloroplast divisions in algae are legion. In 1964 Green reported cinematic observations of chloroplast division in Nitella. He recorded elongation of plastids in the direction of wall strain as the internodal cells elongated, although plastids continued to divide even when the cells were prevented from elongating. Schiff and Epstein (Goodwin, 1966) have reported on the two aspects of plastid continuity in Euglena -- the formation of chloroplasts from proplastids being separate in time from the replication by division of the mature chloroplasts. In Acetabularia mediterranea (Shepard,

1965) the chloroplasts continue to divide even when the nucleus is removed, and plastids show an increase of 50-100% in two weeks. Chloroplast (or chromatophore) divisions have also been reported for brown algae (Evans, 1966; von Wettstein, 1954), red algae (Mitrakos, 1960; Bouck, 1962) and Chromulina which has one nucleus, one mitochondrion, and one plastid per cell all of which divide together when the cell divides (Manton, 1959). Chloroplasts have been observed to divide in many other algae as well.

In Anthoceros (Davis, 1899) the chloroplast is transmitted from cell to cell by plastid division at the time the cell divides. A similar division, but of the proplastid, is reported for the lycopod Isoetes (Stewart, 1948). In the gametophyte of a fern, Matteuccia struthiopteris, Gantt and Arnot (1963) observed a type of chloroplast division in which the inner membrane of the chloroplast double bounding membrane moved across the plastid separating it into two parts. Most other plastid divisions involve a simple fission in which both membranes pinch in, although Frey-Wyssling and Muhlethaler (1965) have reported division of Solanum nigrum mature plastids by the cutting across of the inner membrane only.

In the angiosperms, plastid division has been reported for Elodea canadensis (Guilliermond, 1941; Buvet, 1958), Chrysanthemum (Lance, 1958), Conandron (Kusunoki and Kawasaki, 1936), and Solanum nigrum (Frey-Wyssling and Muhlethaler, 1965). Although

for Chrysanthemum and Solanum apparently dividing mature chloroplasts are pictured, it is necessarily the proplastids in the meristematic cells which are transmitted from cell to cell with division of the meristematic cells in the higher plants (Kirk and Tilney-Bassett, 1967).

Besides plastid divisions, further evidence for at least partial autonomy of chloroplasts lies in the fact that they possess both DNA and RNA (Gibor and Granick, 1964; Swift, Kislev and Bogoard, 1964; Goodwin, 1966; Kirk and Tilney-Bassett, 1967). Plastid DNA has been shown to replicate at a different time than the chromosomal DNA of the cell (Chiang and Sueoka, 1967), and it has been reported that in certain marine algae most H^3-thymidine label is incorporated in plastids just before plastid division, indicating DNA synthesis just before plastid division (Steffenson and Sheridan, 1965).

Although most evidence supports the idea of plastid continuity, Bell, Frey-Wyssling and Muhlethaler (1966) have reported that in the egg cell of the fern, Pteridium aquilinum, all plastids and mitochondria are lost and new organelles are formed from the nucleus before fertilization. This report is questioned by Kirk and Tilney-Basset (1967) on the basis of the greater evidence for plastid continuity as reported for other plants. Such evidence includes plastid divisions and the presence of nucleic acids in plastids as well as a report of continuity of plastids in the egg cell of another fern, Dryopteris (Menke

and Fricke, 1964). It might also be noted that in Euglena once the cells have been permanently bleached by streptomycin, the cells do not develop more plastids (Provasoli, Hutner and Pinter, 1951). The nucleus alone cannot direct the formation of more plastids. In general the theory of plastid continuity is considered to be still valid.

The relationship of mitochondria and plastids must also be considered as they are similar in many ways and both contain nucleic acids and are at least semiautonomous (Gibor and Granick, 1964). Weier (1963), however, has pointed out that the mitochondrial structure is much more stable between species than is the plastid structure. Wildman, Hongladarom and Honda (1962) reported cinephotomicrographic evidence of "mitochondria" developing from blebs pinched from chloroplasts of spinach. These blebs, although the size of mitochondria, were not shown by cytochemical tests to be functioning as mitochondria. In 1968 Maier and Maier reported the development of mitochondria-like blebs from plastids in Polytrichum. These blebs, however, stained as plastid material rather than mitochondria. As far as is known mitochondria and chloroplasts, at least in the mature state, are separate and distinct organelles.

Considering the similarities of plastids to prokaryotic cells in that the plastids are self replicating, nucleic acid containing entities which divide by fission, it is not surprising that plastids are sometimes considered to have evolved from ancient prokaryotic symbionts.

Altmann (Kirk and Tilney-Bassett, 1967) suggested this in 1890, and more recently Ris (1961) and Ris and Plaut (1962) have suggested that modern evidence may also point to these organelles as former symbionts. Some genes controlling the plastids are obviously in the nucleus, but Kirk and Tilney-Bassett (1967) suggest that these controlling genes may have evolved as the symbionts became regular parts of the cell.

In order to study dividing chloroplasts one must select a suitable plant to use for observations. The plants selected for these studies, gametophytes of Equisetum telmateia Ehrh. and E. kansanum Schaffner, have been described by Walker (1921, 1931) as follows: In E. kansanum the first cell division forms rhizoid and thallus cells which are followed by a filamentous growth developing into a massive tissue. The thallus base shows little chlorophyll and supports upright, columnar green branches several cells thick with meristematic regions at the tips. Antheridia and archegonia develop from the meristematic tissue and alternate until formation of sporophytes stops growth of the gametophytes. The mature thallus is lobed and has a radial form. Equisetum telmateia follows a similar growth pattern, and antheridia and archegonia alternate on the thalli. However, the gametophytes develop flat, laminate plates of cells rather than columns. The growth of E. telmateia gametophytes in microculture is described by Jones and Hook (1970).

Very little is in the literature concerning Equisetum chloroplasts, but Manton (Goodwin, 1966) has shown chloroplasts in a young developing spore of E. limosum. These plastids contain starch and have some internal membrane structure but lack organized grana and are much smaller than the plastids of the sporangial wall. She has also illustrated certain chloroplasts in the sporangial wall which contain large crystalline inclusions as well as starch.

MATERIALS AND METHODS

Spore Collection and Storage

Spores were obtained from strobili of Equisetum telmateia Ehrh. (Figure 1-1), collected along roadsides and railroad tracks in the Coast Range and Willamette Valley of Oregon in late March and early April, and from strobili of Equisetum kansanum Schaffner (Figure 1-2), collected by a roadside near St. George, Kansas in late June. All strobili were selected when just beginning to shed spores and were spread on paper and allowed to dry and dehisce for three to four days under room conditions. Shed spores were recovered and placed in screw-cap jars to form a layer about two to three cm thick. They then were covered by a three cm thick layer of glycerine, and placed in a freezer at -10°C. Spores remained viable under these conditions for at least three years (Jones and Hook, 1970). These germinated and grew to maturity when exposed to light and moisture on a simple mineral nutrient medium with no added carbon source. Spores stored dry at -10°C remained viable for five to six months and also were used occasionally.

Media

The medium used has 200 ml Hildebrandt's stock minerals (Hildebrandt, Riker and Duggar, 1946) and 40 mg ferric tartrate in

800 ml distilled water. Iron chelate was sometimes substituted for the ferric tartrate by using five ml/L of the following stock: 0.746 g Na_2-EDTA, 0.556 g $FeSO_4 \cdot 7H_2O$, and 100 ml of distilled water. The type of iron used did not appear to effect growth in any way. For petri plate cultures, six g/L agar was added to the nutrient medium.

Preparation of Cultures

Both microcultures and agar plates were seeded with viable Equisetum spores. If the spores had been stored under glycerine they were washed by placing a small mass of spores on filter paper in a funnel and stirring lightly in five to ten ml mineral nutrient solution. The filter paper with spores then was spread in a petri plate, and the spores were kept moist until used. Microcultures (Jones et al., 1960) were made as follows: A drop of mineral oil was placed at each end of a standard microscope slide. Square 22 x 22 mm cover slips were lowered onto the drops to form "risers." When fixatives were to be injected into the culture chambers the "risers" were made two coverslips thick to permit the entry of the injecting needle. Next a small droplet of nutrient medium was placed between the two risers. A few fibers of the wet filter paper with spores attached were picked up with a teasing needle and touched to the drop of liquid nutrient medium in the center of the slide leaving behind five to 20 spores. A 22 x 40 mm cover slip was placed over the drop, and the area

remaining under the cover slip was sealed with mineral oil. This prevented evaporation of the media and allowed exchange of gases. Prior to being used, the mineral oil was baked at 60°C for two weeks to remove any volatile toxins and then was autoclaved.

Petri plates with mineral nutrient agar were inoculated with spores remaining after each group of microcultures had been made. Both the agar plates and the microculture slides, each within a petri plate, were placed in clear plastic refrigerator boxes under fluorescent lights. Light intensities inside the boxes varied from 150-300 foot candles, and either constant illumination or a 16-hour photoperiod was maintained. Temperatures ranged from 16-26°C.

As contaminants were introduced with the spores, none of the cultures remained free of bacteria and/or fungi. However, contamination was kept at a minimum by using sterile techniques whenever possible. The gametophytes grew best when the liquid mineral nutrient was not autoclaved. Some contaminants, especially species of Penicillium, appeared to enhance growth of Equisetum gametophytes.

Light Microscopy

Microcultures were examined using bright-field illumination primarily, and many observations were recorded photographically. For chloroplast observations, a blue Wratten C5 filter or dark

phase-contrast optics were used to get optimum contrast between plastids and the background. Heat absorbing glass was used to protect the living gametophytes. Standard photomicrographs were taken with a Polaroid MP-3 Land Camera and Polaroid roll film, ASA 3000. For motion pictures, an H-16 Rex Bolex camera with Kodak Tri-X reversal film was used. Movies of Equisetum growth and cell division were made at standard speed (12 frames per second) or at four-second time-lapse. Time-lapse movies of chloroplast divisions were made using 12 or 20 second intervals.

Electron Microscopy

Gametophytes in microcultures were fixed by injecting 3% glutaraldehyde in 0.07 M diphase phosphate buffer, pH 7, into microculture chambers while the slides were being observed with a light microscope. Cytoplasmic streaming stopped instantly when the fixative reached the gametophytes. The fixative, since it mixed with the culture medium, contained about 1% glutaraldehyde when fixation took place. After injection, the slides were placed in the refrigerator at 4°C for four hours with care being taken to prevent evaporation, since insertion of the needle and injection usually broke the oil seal of the microculture. Then the top cover slips were carefully removed, and a drop of warm 2% agar buffered with the same buffer used for the fixative and stained with blue food coloring was placed over the gametophytes. The blue color helped locate the small drops of

hardened agar as the specimens were being placed in plastic vials of buffer where they remained overnight at 4°C.

Gametophytes from agar plates were removed on small blocks of agar and fixed in 3% glutaraldehyde in 0.07 M diphase phosphate buffer, pH 7, for four hours at 4°C. Gametophytes that floated away from the agar during fixation were embedded in separate agar drops, then both preparations were placed in the phosphate buffer overnight.

All specimens were postfixed with 1% osmium tetroxide, again using the pH 7 phosphate buffer, for one hour and were rinsed briefly with the same buffer. From this point a standard dehydration and embedding schedule was used as follows:

50% ethanol	30 min	4°C
70% ethanol	30 min	4°C
95% ethanol	60 min	4°C
100% ethanol	60 min	to room temperature
100% ethanol	60 min	
100% ethanol	60 min	
propylene oxide	20 min	
propylene oxide	20 min	
propylene oxide-Araldite 2:1	12 hrs	
propylene oxide-Araldite 1:2	12 hrs	
Araldite	24 hrs	- flat embedded in aluminum pan
Cured at 60°C	24 hrs	

The Araldite was mixed as follows:

Araldite 6005	13.5 ml (plastic)
DDSA	11.5 ml (hardener)
DMP-30	0.5 ml (accelerator)

The long times involved in the embedding schedule were used to insure adequate dehydration and infiltration of the agar blocks and their enclosed specimens.

Embedded gametophytes were mounted in the desired orientation on plastic capsules. Sections were cut with glass or diamond knives on a Servall "Porter-Blum" MT-1 ultramicrotome and were placed on collodion or Formvar-coated 100 mesh or slotted grids. They were observed with a Philips EM-300 electron microscope at 60 KV.

RESULTS

General Growth of the Gametophytes

The first observable activity in moistened spores in microculture is a churning or rotation of the cytoplasm which is detectable in the time-lapse movies about seven to 14 hours after the spores are placed in culture. This activity may be associated with the first cell division which forms the rhizoid initial and the first thallus cell. The non-germinated spores (Figure 2-1) do not noticeably increase in size until the walls are forcefully shed -- about 14-24 hours after the spores are put into culture. Immediately after the wall is shed elongation of the rhizoid begins, making the entire structure pear shaped. The rhizoid has little noticeable cytoplasmic streaming until it is about 24-30 hours old and 200-400 μ long (Figure 2-3, 4). It remains unicellular and elongates at the tip only.

The thallus cell divides during the first 24 hours so that an average day old gametophyte consists of two thallus cells, densely packed with chloroplasts, and one rhizoid in which there is active visible cytoplasmic streaming.

The gametophytes continue to grow at the rate of one or two new thallus cells per day (Figures 2-5 to 2-9). A typical one week old gametophyte has seven to 14 thallus cells and one rhizoid. Additional rhizoids (Figure 3-1) are formed, beginning when the gametophytes are

about five days old, through division of a thallus cell followed by elongation of one daughter cell.

At the time the gametophytes are one week old, E. telmateia (Figure 3-1) becomes more favorable for use in microcultures as it forms more laminate plates of cells than does E. kansanum. Such a plate of cells is shown at a higher magnification in Figure 3-2. These plates form by a series of cell divisions occurring in rapid succession along the margin of an existing filament or plate. A mass of small compact cells develops. These cells then elongate, sometimes within a single day, to form the new laminate plate which continues to grow by cell divisions near its tip.

A similar series of rapid divisions takes place in the formation of antheridia. Sometimes the two phenomena are combined with developing antheridia being lifted up by the elongation of cells beneath them to form a new laminate plate.

Meristematic regions exist at the tips of growing filaments and in patches along the edges of the laminate plates. The meristematic cells are small and have dense cytoplasm and large nuclei, usually supported in the center of the cells by cytoplasmic strands. It is these cells and those just below them which most frequently divide and in which dividing plastids are most likely to be found.

A cell which will soon divide can be identified by the granular appearance of the centrally located nucleus and the position of many

chloroplasts which are located along the supporting cytoplasmic strands, accentuating the strand's appearance of being "spokes in a wheel." The length of prophase has not been determined accurately due to the difficulty in seeing clearly within the chloroplast-packed cells, although an accurate criterion for prophase is a cessation of cytoplasmic streaming within the cell. This condition apparently exists here for less than an hour before metaphase. Metaphase (Figure 3-3) lasts eight to nine minutes and anaphase, two to three minutes. Anaphase is accompanied by a rocking of the spindle and agitation of the cytoplasmic strands, and is therefore detectable even when the chromosomes are not clearly visible. Cell plate formation begins about three minutes after the chromosomes have separated and usually the new wall is completed (Figure 3-4) within 20-30 minutes after the cell plate first appears. A doughnut shaped phragmoplast precedes the wall across the cell, and the chloloroplasts in the path of the advancing wall are pushed aside. Daughter nuclei are reconstituted within 18-20 minutes after anaphase and before the cell plate is completed.

Formation and maturation of gametangia indicates a condition of maturity of the gametophytes in microculture, and few cell divisions occur in the thalli after this time. Developing antheridia are first noted in microculture when the gametophytes are 10-14 days old, although some form after ten or more weeks. In large, rapidly

growing gametophytes, many antheridia form consecutively so that many stages of development are visible at one time (Figures 4-1, 2, 3). A more common occurrence is the formation of single antheridia on smaller, usually more crowded, gametophytes (Figure 4-4). In the formation of an antheridium, a series of rapid cell divisions forms a central cluster of small, compact cells within which the sperm develop. The sperm at first are quite granular and later become oval, rounded structures which are ready to be released approximately one month after antheridial initiation. Not all antheridia release their sperm in microculture. In some, mature sperm can be seen swimming within the antheridium but are unable to break out. In others (Figure 5-1) sperm are released in a gelatinous matrix. They then become motile and swim in random spirals within the microculture chamber. There is no evidence of their being attracted to any particular objects or substances in the microcultures, but sperm release was not observed on those slides with archegonia. The sperm remain motile for five to seven hours after release in the microcultures. Sperm release from antheridia on gametophytes growing on mineral agar in petri plates follows the same pattern.

Archegonia form rarely in microculture. Those seen were first noted when the cultures were four weeks old (Figure 5-2). They matured and the neck cells opened when the cultures were eight weeks old (Figure 5-3).

No young sporophytes have developed in microculture. However, in the agar plates, where development parallels that in the microcultures, young sporophytes commonly do develop (Figure 5-4). They are first visible about two months after the spores are planted on the agar.

Equisetum gametophytes on a given slide vary in growth patterns and degree of maturity. The thalli are uniformly smaller on the more crowded slides. Most do not live longer than three to four months in microculture, but some gametophytes still show cytoplasmic streaming and a few meristematic cells after nine months in culture. In microculture, as has been mentioned, growth usually does not continue after the formation of gametangia.

Senescent cells appear first near the base of the gametophyte where the oldest cells are located. Senescence is sometimes noted in these cells when the cultures are only 15-20 days old and before developing gametangia have become mature. Although senescent cells often remain alive for several weeks, none have been seen to divide. Growth of the gametophytes is entirely from the meristematic regions at the advancing margins or tips of the thallus. The senescent cells, as seen with the light microscope, appear quite vacuolate with shrunken chloroplasts embedded in a thin layer of parietal cytoplasm; the nucleus is scarcely detectable.

Two types of abnormal conditions are seen in the gametophytes

in microculture. The first is characterized by a stunting of the young gametophytes, especially the rhizoid, and is recognizable shortly after germination. This is apparently the result of improper spore storage. Although some of these stunted gametophytes develop sufficiently to form antheridia, none form flat cells adequate for use in chloroplast studies. The second type of abnormality is characterized by a slowing of growth and the occurrence of chloroplast abnormalities in the older gametophytes -- usually those one to three weeks old. The plastids become shrunken and are extremely vacuolate (Figure 19-1). This condition seems to be related to an excess of bacteria growing in the microcultures. No gametophytes have recovered from this state to mature normally.

Behavior of Chloroplasts

A typical chloroplast in a young Equisetum gametophyte cell is an oval to dumb-bell-shaped structure eight to 12 μ long. Starch grains can usually be seen in each plastid, and grana are detectable at high magnifications.

The crowded chloroplasts in the non-germinated spores and the very young thallus cells are nearly impossible to resolve with the light microscope. Therefore, one of the earliest changes which can be observed is in the growing rhizoid where the chloroplasts begin to shrink.

When the rhizoid first appears (Figure 6-1) the chloroplasts are about the same size as those of the green thallus cell. The rhizoid plastids remain normal in size during the first day (Figures 6-2, 3) but soon begin to shrink, and at 38 hours old (Figure 6-4) they are noticeably smaller. Active cytoplasmic streaming usually begins in the rhizoid shortly before the onset of the chloroplast shrinkage. Occasionally a rhizoid has been observed in which the plastids did not shrink, but in all cases the thallus cells attached to such rhizoids have been dead or injured and apparently non-functional. A typical healthy gametophyte three days old shows a striking difference between thallus cell plastids (Figure 6-5) and rhizoid plastids (Figure 6-6). Chloroplast shrinkage also occurs in secondary rhizoids formed by division of thallus cells (Figure 8-1), but this shrinkage occurs during the first 24 hours as the rhizoid is elongating.

The most striking thing noted concerning chloroplast behavior in Equisetum gametophytes has been the division of the mature chloroplasts in the young growing cells. Division of chloroplasts is best studied by combining plastid counts made on consecutive days with time-lapse movies. Due to the constant shifting of the plastids in the living cells and the long time involved a complete plastid division has been actually watched first hand in the living material only once.

All chloroplast divisions detected have taken place in young, growing tissues and have been found almost exclusively in cells which

divided during the previous 24-48 hours. The smallest, youngest cells at the apex of a thallus lobe usually cannot be used for such work as they are too dense and the plastids are not individually visible. Cells just back of the growing tip, such as the cell indicated in Figure 3-2, are ideal. In such cells the number of plastids increases by 12-50 during the 48 hours following cell division, with a few divisions taking place during the following days. Table 1 summarizes the plastid count information.

The division of a single plastid from one oval to two daughter plastids requires from eight to 30 hours (Figure 7). Division is always accomplished by a pinching-in at the center of the plastid which usually results in two daughter plastids equal in size although occasionally a small portion appears to pinch off one edge or from between the two daughter plastids as they separate. Division sometimes results in two plastids that are each temporarily flattened on the inner side where they have parted, and occasionally the two daughter plastids continue to move together in the cytoplasmic flow after they have apparently become completely separate. They then break apart suddenly and move in different directions. Usually, however, the plastids move away from one another as soon as the division is complete. The pleomorphic plastids assume many shapes before, during, and after division and often become slightly angular when crowded within a cell after a number of plastid divisions have

Table 1. Increase in Equisetum gametophyte chloroplast number after various time periods following cell division.

	Type of cell	Original number plastids	12 hr.	24-30 hr.	48 hr.	3 days	4 days	Total increase
1.	Daughter	43		64				21
2.	Daughter[1]	26		53			60	34
3.	Daughter[2]	36	50	57				21
4.	Daughter[2]	38	61	65				27
5.	Daughter[3]	50		62				12
6.	Daughter[4]	45	45			60		15
7.	Daughter	51		65	91	91		40
8.	Daughter	62		85	104			42
9.	Prophase	85		108[5]	108			23
10.	Prophase	130		179[5]				49
11.	Daughter[6]	57		107				50

[1] Cell shown in Figure 3-2.
[2] Cells shown in Figure 3-3, 4.
[3] Gametophyte crowded, small.
[4] Cell had abnormally small plastids.
[5] Total for two daughter cells.
[6] Cell probably a product of recent division but division not observed. Cell shown in Figure 7.

occurred.

There is no evident synchrony of plastid division. Except for the fact that most occur within 48 hours following cell division, the plastid divisions do not seem to be correlated with any particular cellular structure, function, or position. It should be noted, however, that most plastid divisions do occur in rapidly elongating cells, although some also take place in cells showing little detectable increase in size. Division of any one plastid appears to be independent of all other plastid divisions within a given cell.

No organelles which could be interpreted as proplastids have been seen in any of the gametophytes examined.

The length of time elapsed before a daughter plastid divided again was not determined, but it is necessarily longer than 30 hours -- the longest period of time individual chloroplasts were watched and checked for re-division.

The dumb-bell shape of many plastids does not necessarily indicate that plastid division is taking place. For instance, no divisions were detected in the dumb-bell shaped plastids in the elongated cells shown in Figures 8-2, 3. Eventually these plastids became senescent and shrunken (Figure 8-4) without entirely losing their dumb-bell shape.

Chloroplasts have been seen to fuse in only two instances, both recorded on time-lapse movie film. In both cases, the two plastids

which fused were daughter plastids which had just formed by a very rapid (eight hour) division of an existing plastid which was already smaller than most others in the cell. The division appeared to be complete or nearly so and was immediately followed by a refusion of the two daughter portions (Figures 7-12 to 16).

Giant plastids are occasionally found as seen in Figures 8-2, 3, 4. These have never been seen forming or dividing, and they may be the result either of failure of plastids to divide or of fusion. The giant plastids in Figure 8-4 remained large until the cell became senescent and died.

Chloroplasts in the vicinity of developing antheridia tend to become shrunken so that the jacket cells surrounding each antheridium appear to be quite empty by the time the antheridium is mature. The effect is most striking in young gametophytes where plastid size can range from only three μ in the jacket cells to 10 to 12 μ only three cells away. Note the decrease in size of the chloroplasts as you progress from cells 1 to 2 to 3 in Figure 9.

Ultrastructure

The typical mature non-dividing chloroplast in the young cells of Equisetum gametophytes (Figure 10) is a double membrane bound organelle containing grana, which consist of stacks of two to many thylakoids; fretwork; background stroma; starch grains; and

osmiophillic granules. The starch grains generally appear in the electron microscope sections in the center of open spaces as if partially eroded. The plastids have no pyrenoids. Each chloroplast within a healthy cell is partially enclosed by a sheath of endoplasmic reticulum. No crystalline material is seen in the gametophyte chloroplasts.

Non-germinated spores did not embed sufficiently well to be used for extensive ultrastructure work, but those few sections which were obtained showed fully formed, mature chloroplasts within the non-germinated spore.

In recently germinated (14-22 hours old) gametophytes (Figure 11-1) the chloroplasts also have the typical appearance of mature plastids. No proplastids are seen. The thallus cell cytoplasm is dense, with many small vacuoles. Plastids are scattered throughout (Figures 11-2, 3). There are many mitochondria, dictyosomes, and a large population of polyribosomes. Thallus cell ER is limited largely to that found in the plastid sheaths. At this age chloroplasts have the same size and appearance in both thallus and rhizoid cells. The rhizoid cytoplasm is similar to that of the thallus although it rapidly becomes more vacuolate, especially at the basal end of the rhizoid near the green cell. Rhizoid cytoplasm (Figure 11-4) contains fewer polyribosomes than that of the thallus but has much ER that is not associated with plastids. The rhizoids have more dictyosomes near

their growing tips than basally, and possibly a total of more dictyosomes than are in the thallus cell. Mitochondria are similar in appearance within the two cells. Both cells contain "gray bodies" (Figure 11-2) which appear to be lipid globules and do compare in size and form with lipid-positive drops detected by Sudan IV tests run on intact gametophytes. (Lipid drops in a non-killed gametophyte can be seen in Figure 6-5.) In the young cells, lipid is apparently also found as black crescents against the edges of some vacuoles (Figures 11-2, 3).

In 26 hour old cultures a second green cell has formed and the thallus cell cytoplasm is becoming more vacuolate (Figures 12-1, 2). At this time some size difference is detectable between the chloroplasts of the thallus cell and the rhizoid (Figures 12-3, 4). The rhizoid plastids, however, maintain an organized internal membrane system with starch and still possess an ER sheath.

Thirty-nine hours after culturing, the green cells are larger and highly vacuolate (Figure 13-1). The rhizoid near the green cell has a large vacuole surrounded by parietal cytoplasm containing the organelles (Figures 13-1, 4). The cytoplasm near the tip of the rhizoid, however, remains dense (Figure 13-3). By this time the size difference between chloroplasts in the thallus and rhizoid cells is more pronounced (Figure 13-2), but the rhizoid plastids still have an organized internal membrane system and in most cases an ER sheath.

After four days of growth (Figure 14-1), the cytoplasm of the

thallus and rhizoid cells differs markedly. Rhizoid cytoplasm contains many more small vacuoles, blebs, dictyosomes, and much more ER not associated with plastids than does that of the thallus cell. Thallus cell cytoplasm contains fewer but larger vacuoles, many polyribosomes, and some dictyosomes. Endoplasmic reticulum is associated with plastids in all thallus cells, and in some there is very little ER not in the plastid sheaths. Mitochondria are similar in size, shape, and number in the rhizoid and thallus cells. Intercellular connections are found both between rhizoid and thallus cells and between the various thallus cells. The differences between rhizoid and thallus cell plastids have become extensive by this time with the rhizoid plastids being quite small, degenerate, and largely lacking in internal membrane structure and ER sheaths (Figures 14-1, 2).

Although sectioned cells from the very young gametophytes are ideal places to find dividing chloroplasts (to be discussed in a later section), the cells when alive are so dense that individual plastids can scarcely be distinguished with the light microscope. However, by the time the gametophytes are one week old chloroplasts in the cells are separated enough so that individual plastids are visible with light microscopy, and light and electron microscopy can be more easily coordinated. In order to have such coordination, it is necessary to be able to distinguish the type of cell (its degree of maturity, etc.) being observed with the electron microscope.

Non-dividing cells in meristematic regions at the tips of the thalli (Figures 14-3, 4) have central nuclei supported by cytoplasmic strands and chloroplasts in the parietal cytoplasm and occasionally along the strands. A cell which will soon divide, however, has many plastids along the supporting cytoplasmic strands, mostly oriented toward the nucleus (Figure 15-1). Most stages of mitosis were not seen on any of the electron microscope sections, but cell plate formation is shown in Figures 15-2, 3. Few dictyosomes are seen near the forming cell plate, but many vesicles, which may be from dictyosomes, are present, and the cell plate itself appears to form from an aggregation of these small blebs.

As the gametophyte continues to develop, antheridia often form at the growing tips. A section of a gametophyte two weeks old is shown in Figure 16 with a young antheridium in the left lobe. Cell divisions take place rapidly in this area. Another growing point at the tip of the right lobe has compact vegetative, meristematic cells. Both these cells of the growing point and the cells near the antheridium are areas in which dividing plastids may be found. The lower portion of the gametophyte contains elongated cells which are no longer dividing, and has plastids which also are not dividing and which will soon become senescent.

A growing gametophyte tip may produce several antheridia with sperm in different stages of development (Figures 17-1, 2). The

chloroplasts of the antheridial jacket cells, while smaller than those of the regular thallus cells, are still considerably larger than the seemingly degenerate chloroplasts in the sperm mother cells (Figure 17-3). Although the plastids do degenerate in the developing sperm, they are still recognizable in sperm which appear mature and ready to be released (Figure 17-4). Internal membrane systems are virtually nonexistent, but the plastids can be identified in the sperm by the presence of starch grains, and they remain surrounded by ER sheaths. In the mature sperm the plastids with starch grains are scarcely larger than the mitochondria.

Plastid degeneration in senescent cells (Figure 18) follows a different pattern. Large amounts of starch may or may not be present. The internal membrane system remains somewhat intact, but the plastids become smaller and the ER sheath is lost. In senescent cells the nucleus is likely to become lobed. Eventually vacuolar membranes in these cells break down.

Another type of plastid degeneration takes place in cells suffering from heavy bacterial contamination in the microcultures. It appears in light microscopy as small bodies forming within the plastids (Figure 19-1). Electron microscopy, however, shows that these plastids are becoming highly vacuolate (Figure 19-2). Eventually the plastid degenerates completely losing internal membrane structure and becoming a mass of vacuoles (Figure 19-3).

Gametophytes containing plastids of this type do not grow and soon die.

Plastids in stages of division have been found in the cells of very young gametophytes and in the meristematic regions at the tips of older gametophytes.

When sectioned parallel with the cell wall the plastids in the parietal cytoplasm appear as shown in Figure 20. This paradermal view is comparable to the light microscopy views shown in Figure 7. The grana for the most part lie parallel with the section and so appear as somewhat blurred dark areas. The fretwork is seen in various patterns depending on the angle at which it lies in the cell and on the plane of sectioning. The dividing plastids are bound in the usual way by a double membrane and possess an ER sheath, starch, osmiophillic granules, and normal stroma. Division of the plastids progresses from a slight central constriction (Figure 20-1) to a more advanced pinching-in (Figures 20-2, 3) with no dividing membrane forming across the narrowed central area. The internal membranes are apparently pushed aside as the plastid double bounding membrane constricts.

In plastids sectioned at right angles to those shown in Figure 20 the grana are seen as the usual stacks of thylakoids. The central constriction seen in the paradermal view is shown in this plane also, indicating that the plastids constrict from all sides. The internal membranes may be either compressed (Figure 21-1) or apparently may

be severed (Figure 21-2). The positions of the internal membranes may vary from an orderly arrangement (Figure 22-1) to one in which one or more grana are tipped at right angles in the area of the constriction (Figure 22-2), perhaps as a result of crowding of the membranes in this area.

In some cases the plastids are apparently twisted as shown in Figure 23. The internal membranes in the two halves may lie at right angles (Figure 23-1) or the membranes of the central portion may lie at different angles than those at the ends of the plastid (Figure 23-2). Such plastids are often found with one portion against the cell wall in the parietal cytoplasm and the other in one of the cytoplasmic strands supporting the nucleus.

Another item of interest concerning plastid division is that time-lapse movies sometimes show small blebs of plastid material being nearly left behind as two daughter plastids pull apart. Figure 24-1 shows a plastid which is dividing but has a starch grain near the center. Such a situation might cause formation of a bleb as the daughter plastids pull away from the area surrounding the starch.

Two daughter plastids occasionally continue to travel together for a short time after division and then suddenly break away and travel in different directions. Figure 24-3 indicates a condition which might explain this. The ER sheath so completely encloses the two halves of the nearly divided plastid that the two daughter plastids might

be held together for a time after division.

Figure 24-2 shows a type of apparent plastid division sometimes seen in cells near antheridia. These plastids, often somewhat smaller than those of the regular thallus cells, appear to be dividing in a longitudinal plane. Since the cells around the antheridia are too dense to be examined closely with light microscopy and no movies have been made of them, it is not possible to say whether the plastids are actually dividing in this plane or only appear to be as a result of pleiomorphism. This particular plastid shape is, however, characteristic of some plastids in antheridial jacket cells and in sperm mother cells. It has not been seen in other cells of the thallus or in the rhizoids.

One final matter concerning the chloroplasts and their possible role in the cell should be discussed. In the thallus cells of the very young (one or two day old) gametophytes, structures are found about the size of mitochondria but with internal contents which more closely resemble chloroplast stroma (Figures 25-1, 3). Such structures are more common in some gametophytes than others, and are seen more often in one to two day old organisms than in the just germinated spores. This, along with the fact that mature plastids are known to be present in the spores, would indicate that these blebs are not a form of proplastid. The plastids of the one to two day old gametophytes are much more pleiomorphic than those of older cells. These

irregularly shaped plastids often have tails (Figures 25-1, 2) which are similar in appearance to the structures with contents resembling stroma found free in the cytoplasm. It may be that the stroma bodies are cross sections of attached chloroplast tails or they may be detached blebs. Although such blebs have not been seen to pinch off it should be remembered that chloroplasts in gametophytes this young are too densely packed to be examined in detail with light microscopy. No satisfactory intermediates have been found between these and mitochondria. The stroma bodies are more often seen in thallus cells than in rhizoids.

DISCUSSION

When studying chloroplasts of Equisetum gametophytes one becomes impressed with their pleomorphism. These plastids are capable of assuming an endless assortment of shapes and sizes depending on the circumstances under which they are found. When one also considers that the degree of internal membrane structure varies with the type of cell in which the plastid is found it becomes apparent that however autonomous the plastids may be they are still drastically effected by the environment provided by the cell in which they are found. It is difficult, if not impossible, to prove any plastids completely autonomous as long as they cannot be grown separately from their cells. Until plastids can be grown in pure culture, studies of chloroplasts must always take into consideration the type of cells used and the condition of the cells.

It has been noted that the chloroplasts of Equisetum gametophytes replicate primarily in young, meristematic cells which have just divided and are undergoing considerable elongation. It is, of course, necessary for plastid divisions to take place in these cells if the plastid number is to be maintained in the newly formed cells. The "need" for more chloroplasts, however, does not explain the triggering mechanism or means of plastid division. It has been shown that some plastid divisions occur more than 24 hours after cell division; and, although the plastids do appear to be stretched somewhat

and pulled out toward the nucleus during cell division, the fact that the cell has divided is not necessarily in itself a triggering mechanism for plastid division. There may be some unknown factor, possibly chemical, which relates plastid division to cell division, but such a factor has not been discovered.

Most divisions of Equisetum chloroplasts seem to occur in cells which are rapidly elongating although the divisions are not necessarily oriented in any particular way relative to the direction of wall elongation. Green (1964) showed wall elongation to have an effect on plastid orientation during plastid division in Nitella. The Nitella plastids, however, continued to divide when wall elongation was prevented. Equisetum plastids also will divide, at least to a limited extent, in cells which are elongating very little. It seems unlikely that the strain of cell elongation acting on the parietal cytoplasm is a necessary precursor for plastid division. Since it is possible for the plastids to become so crowded within the parietal cytoplasm of a cell that they become angular as they are pressed together, it also seems unlikely that plastids react in such a way as to space themselves a prescribed distance apart.

Plastid size may be a factor in division as the small five μ plastids which were seen to divide immediately fused again. A typical dividing plastid in Equisetum is 10-12 μ long. Although a study of plastid growth rates was not done, it is assumed that the daughter

plastids (five μ) enlarged to the 10-12 μ size during the 48 hours following cell division and were large enough to divide again by the time the cells divided again.

Not all plastids in a given cell divide, even following a cell division. It may be that a plastid must be "ready" for division -- possibly a matter of DNA being replicated. Various conditions during and after cell division then might trigger the plastid division. This might not adequately explain plastid division taking place more than 24 hours after cell division, however.

The generation time of Equisetum plastids was not determined, but since cell divisions take place rapidly in the meristematic regions -- a daughter cell may divide again in 48 hours -- it is reasonable to assume that the plastids must be capable of keeping up and have a generation time perhaps as short as 48 hours. Possibly the plastid generation time varies with different cytoplasmic conditions. Certainly, in the older, elongated cells plastids did not divide over periods of several days to a week.

The internal structure of the dividing chloroplasts gives little clue as to the mechanism of division. Gantt and Arnott (1963) and Frey-Wyssling and Muhlethaler (1965) reported that only the inner membrane of the chloroplast double bounding membrane invaginated to separate the dividing plastid. There is no hint of this in Equisetum.

The internal membranes of the dividing Equisetum plastids do

lie somewhat twisted, as compared with the non-dividing plastids, perhaps due to a slight twisting of the whole plastid or a constriction of the fretwork and grana as plastid division proceeds.

The matter of the possible relationship between the stroma bodies and plastid tails must also be considered. It has been mentioned that the Equisetum chloroplasts are very pleiomorphic. They are especially so in the very young sporelings. These plastids often have extensions of the stroma which are in internal structure exactly like the stroma bodies which are found in the cytoplasm. A possible explanation for the stroma bodies may be that they are merely cross sections of plastid tails still attached to the plastids which do not appear in the sections observed. It may also be that the stroma bodies have pinched off from chloroplasts. If so the developmental fate of the stroma bodies is not known. Wildman, Hongladarom and Honda (1962) reported cinemicrographic studies of blebs pinching off from and fusing with chloroplasts. They have interpreted some of these as being mitochondria -- an assumption based on their size and shape. In Equisetum there are no clearcut intermediates between the stroma bodies and mitochondria, but it seems likely that the stroma bodies are blebs which have pinched off the plastids as Wildman has shown. Weier and Thomson (1962) found that single-membraned bodies pinched off from the stroma of chloroplasts in plants placed in a confined atmosphere for 48 hours. Most of the Equisetum stroma

bodies appeared about 48 hours after culturing, but the stroma bodies are bound by double membranes. The observation of Maier and Maier (1968) in which mitochondria-like blebs, which nevertheless stained like plastids, were pinched off from the plastids in young sporophytes of Polytrichum moss, may be related. There is, however, no evidence that the stroma bodies develop into chloroplasts. The small, poorly developed plastid in Figure 11-3 could be an intermediate, but this seems unlikely as only two plastids of this type have been detected. This seems a small number of intermediates to explain the fate of the number of stroma bodies seen. Although it is theoretically possible for the stroma bodies to develop into chloroplasts, providing an adequate amount of plastid genetic material is present in them, the fate of the Equisetum stroma bodies may be that of becoming degenerate blebs lost in the cytoplasm.

The chloroplasts of Equisetum gametophytes are pleiomorphic organelles capable of self-replication by fission of the mature plastids. The chloroplasts appear not to form by any other means in the developing gametophyte. Although proplastids may exist at some stage of Equisetum development, perhaps in the very young developing spore, they do not play a part in chloroplast formation in the growing gametophytes. The chloroplasts are sensitive to their cytoplasmic environment and exhibit drastic degeneration in the differentiating rhizoid and antheridial cells.

BIBLIOGRAPHY

Bell, P. R., A. Frey-Wyssling and K. Muhlethaler. 1966. Evidence for the discontinuity of plastids in the sexual reproduction of a plant. Journal of Ultrastructural Research 15:108-121.

Bouck, B. J. 1962. Chromatophore development, pits, and other fine structure in the red alga, Lomentaria baileyana (Harv.) Farlow. Journal of Cell Biology 12:553-569.

Buvat, R. 1958. Recherches sur les infrastructures du cytoplasme, dans les cellules du méristéme apical, des ébauches foliaires et des feuilles développées D'Elodea canadensis. Annales des Sciences Naturelles: Botanique 19:121-161.

Chiang, K. S. and N. Sueoka. 1967. Replication of chloroplast DNA in Chlamydomonas reinhardi during vegetative cell cycle: its mode and regulation. Proceedings of the National Academy of Science 57:1506-1513.

Davis, B. M. 1899. The spore-mother-cell of Anthoceros. Botanical Gazette 28:89-109.

Evans, L. V. 1966. Distribution of pyrenoids among some brown algae. Journal of Cell Science 1:449-454.

Frey-Wyssling, A. and K. Muhlethaler. 1965. Ultrastructural plant cytology. Elsevier Publishing Company, New York. 377 p.

Gantt, E. and H. J. Arnott. 1963. Chloroplast division in the gametophyte of the fern Matteuccia struthiopteris (L.) Todaro. Journal of Cell Biology 19:446-448.

Gibor, A. and S. Granick. 1964. Plastids and mitochondria: inheritable systems. Science 145:890-897.

Goodwin, T. W. (ed.). 1966. Biochemistry of chloroplasts. Vol. 1. Academic Press, New York. 476 p.

Green, P. B. 1964. Cinematic observations of the growth and division of chloroplasts in Nitella. American Journal of Botany 51:334-342.

Guilliermond, L. 1941. The cytoplasm of the plant cell. Trans. L. R. Atkinson. Chronica Botanica Co., Waltham, Mass. 247 p.

Hildebrandt, A. C., A. J. Riker and B. M. Duggar. 1946. The influence of the composition of the medium on growth in vitro of excised tobacco and sunflower tissue cultures. American Journal of Botany 33:591-597.

Jones, L. E., A. C. Hildebrandt, A. J. Riker and J. H. Wu. 1960. Growth of somatic tobacco cells in microculture. American Journal of Botany 47:468-475.

Jones, L. E. and P. W. Hook. 1970. Growth and development in microculture of gametophytes from stored spores of Equisetum. American Journal of Botany 57:430-435.

Kirk, J. T. O. and R. A. E. Tilney-Bassett. 1967. The plastids, their chemistry, growth, and inheritance. Freeman, San Francisco. 608 p.

Kusunoki, S. and Y. Kawasaki. 1936. Beobachtungen über die Chloroplasten teilung bei einigen Blütenpflanzen. Cytologia 7:530-534.

Lance, A. 1958. Infrastructure des cellules du méristéme apical et des jeunes ébauches foliaires de Chrysanthemem segetum L. (Composées). Annales des Sciences Naturelles: Botanique 19: 165-202.

Maier, K. and U. Maier. 1968. Zur Frage einer Neubildung von Mitochondrien aus Plastiden. Protoplasma 65:239-242.

Manton, I. 1959. Electron microscopical observations on a very small flagellate: the problem of Chromulina pusilla Butcher. Journal of the Marine Biological Association of the United Kingdom 38:319-333.

Menke, W. and B. Fricke. 1964. Beobachtungen über die Entwicklung der Archegonien von Dryopteris felix mas. Zeitschrift für Naturforschung 19b:520-524.

Mitrakos, K. 1960. Feinbau und Teilung bei Plastiden einiger Florideen-Arten. Protoplasma 52:611-617.

Naegeli, C. 1846. Bläschenförmige Gebilde im Inhalte der Pflanzenzelle. Zeitschrift für Wissenschaftliche Botanik 3:94-128.

Provasoli, L., S. H. Hutner and I. J. Pinter. 1951. Destruction of chloroplasts by streptomycin. Cold Spring Harbor Symposium of Quantitative Biology 16:113-120.

Ris, H. 1961. Ultrastructure and molecular organization of genetic systems. Canadian Journal of Genetics and Cytology 3:95-120.

Ris, H. and W. Plaut. 1962. Ultrastructure of DNA-containing areas in the chloroplast of Chlamydomonas. Journal of Cell Biology 13:383-391.

Schimper, A. F. W. 1883. Über die Entsicklung der Chlorophyllkörner und Farbkörner. Botanische Zeitung 41:105-113.

Shephard, D. C. 1965. Chloroplast multiplication and growth in the unicellular alga Acetabularia mediterranea. Experimental Cell Research 37:93-110.

Steffensen, D. M. and W. F. Sheridan. 1965. Incorporation of H^3-thymidine into chloroplast DNA of marine algae. Journal of Cell Biology 25:619-626.

Stewart, W. W. 1948. A study of the plastids in the cells of the mature sporophyte of Isoetes. Botanical Gazette 110:281-300.

Swift, H., N. Kislev and L. Bogorad. 1964. Evidence for DNA and RNA in mitochondria and chloroplasts. Journal of Cell Biology 23:91. (Abstract)

von Wettstein, D. 1954. Formwechsel und Teilung der Chromatophoren von Fucus vesiculosus. Zeitschrift fur Naturforschung 9b:476-481.

Walker, E. R. 1921. The gametophytes of Equisetum laevigatum. Botanical Gazette 71:378-391.

_____ 1931. The gametophytes of three species of Equisetum. Botanical Gazette 92:1-22.

Weier, T. E. 1963. Changes in the fine structure of chloroplasts and mitochondria during phylogenetic and ontogenetic development. American Journal of Botany 50:604-611.

Weier, T. E. and W. W. Thompson. 1962. Membranes of mesophyll cells of Nicotiana rustica and Phaseolus vulgaris with special reference to the chloroplasts. American Journal of Botany

49:807-820.

Wildman, S. G., T. Hongladarum and S. I. Honda. Chloroplasts and mitochondria in living plant cells: cinephotomicrographic studies. Science 138:434-436.

APPENDIX

Figure 1-1. Young strobili of Equisetum telmateia.

Figure 1-2. Strobili of E. kansanum.

FIG 1

Figure 2. Spore germination and early development of E. kansanum gamteophytes. 115 X.

Figure 2-1. Non-germinated spores.

Figure 2-2. Eighteen hours old.

Figure 2-3. Twenty-three hours old.

Figure 2-4. Thirty hours old.

Figure 2-5. Fourty-four hours old.

Figure 2-6. Fifty-four hours old.

Figure 2-7. Three days old.

Figure 2-8. Five days old.

Figure 2-9. Six days old.

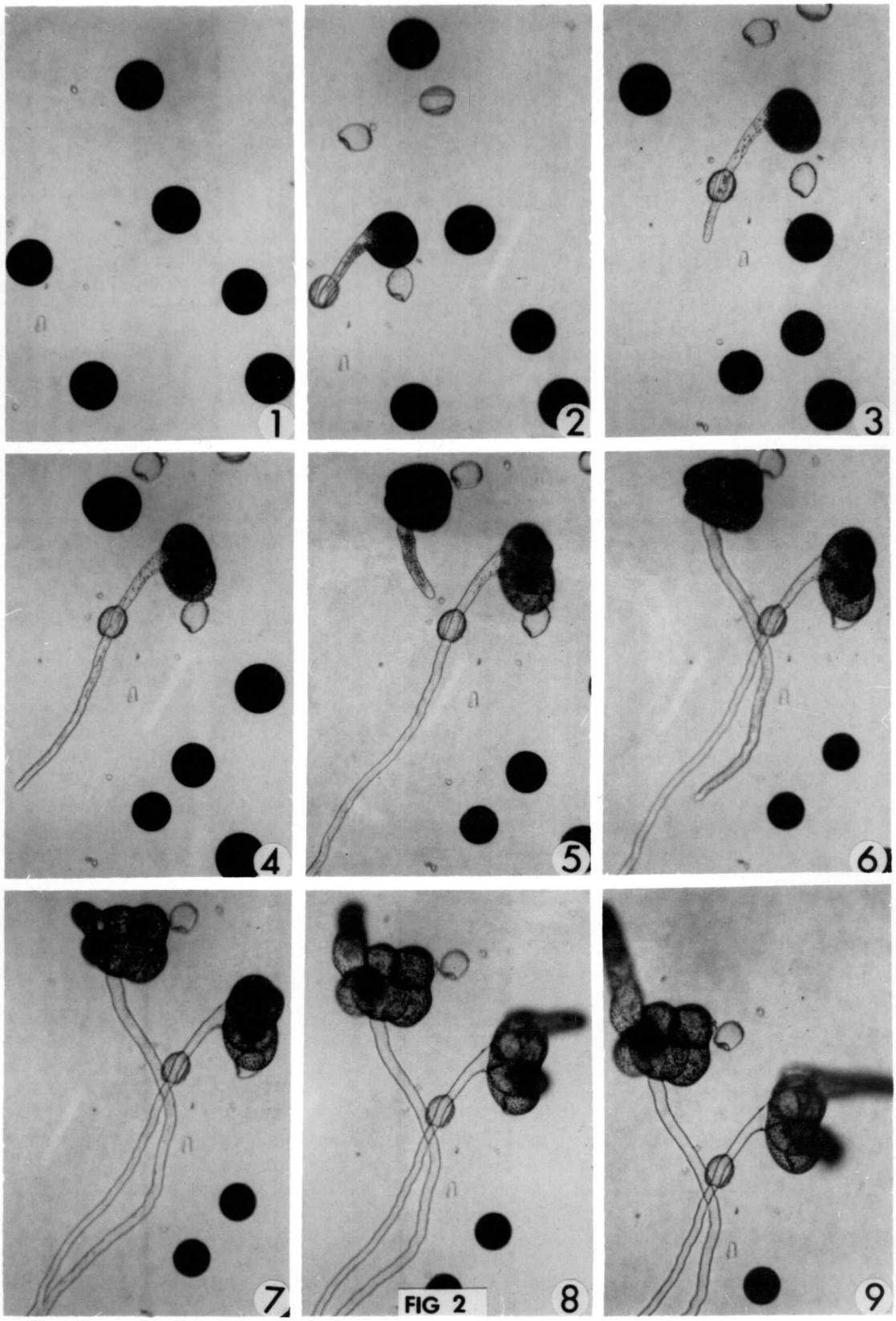


FIG 2

Figure 3-1. Gametophyte of E. telmateia two weeks old showing growth pattern and secondary rhizoids. 95X.

Figure 3-2. Thallus lobe of E. telmateia three weeks old, showing recently divided cell. 295X.

Figure 3-3. Metaphase in cell of E. telmateia thallus. 465X.

Figure 3-4. Daughter cells separated by new wall. Picture taken one hour seven minutes after Figure 3-3. 465X.

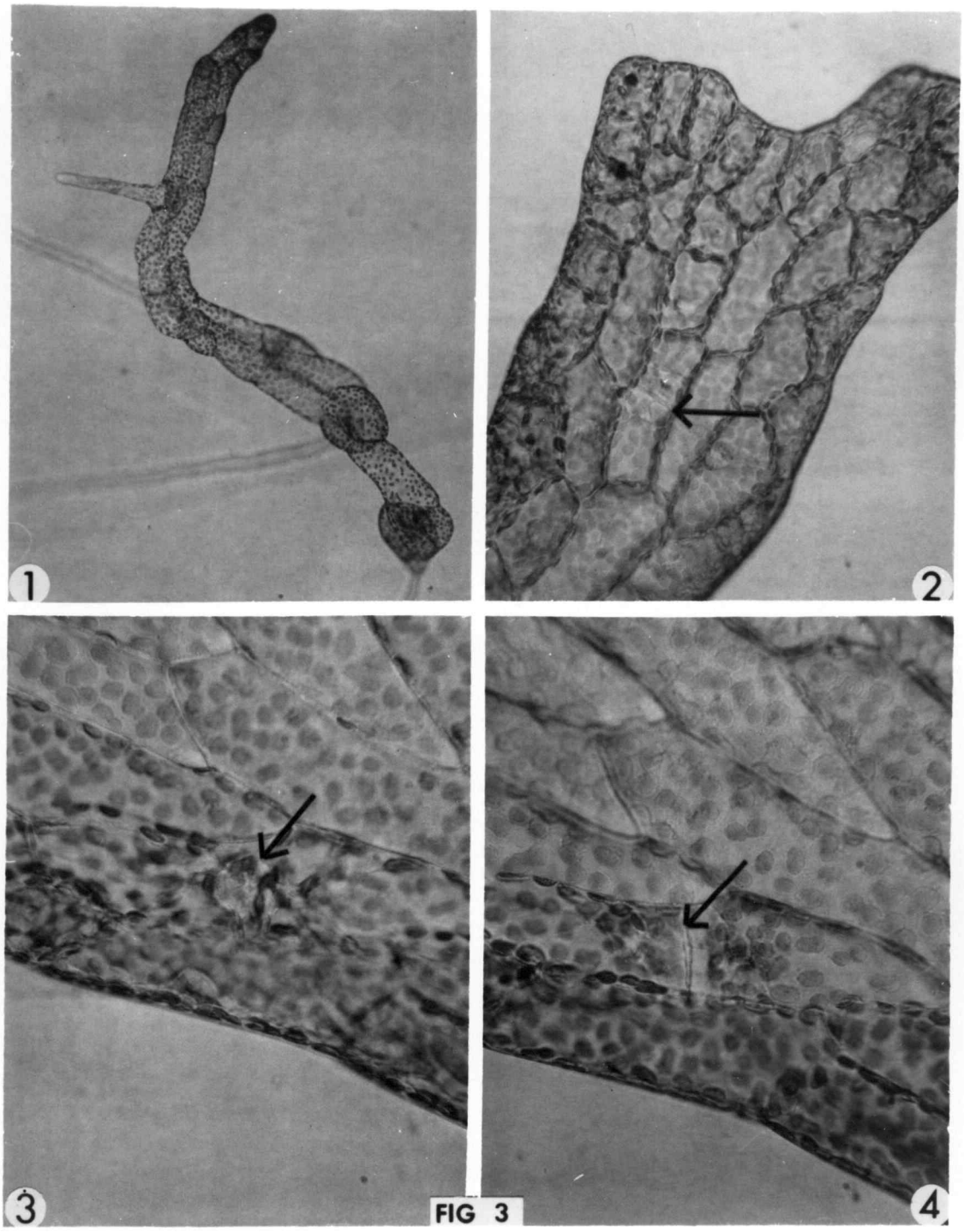


FIG 3

Figure 4-1, 2, 3. Development of antheridia on E. telmateia gametophyte at seven, eight, and ten weeks old, respectively. Note release of sperm from antheridium indicated in Figure 4-3. 115X.

Figure 4-4. Single antheridium on E. telmateia gametophyte four weeks old. 295X.

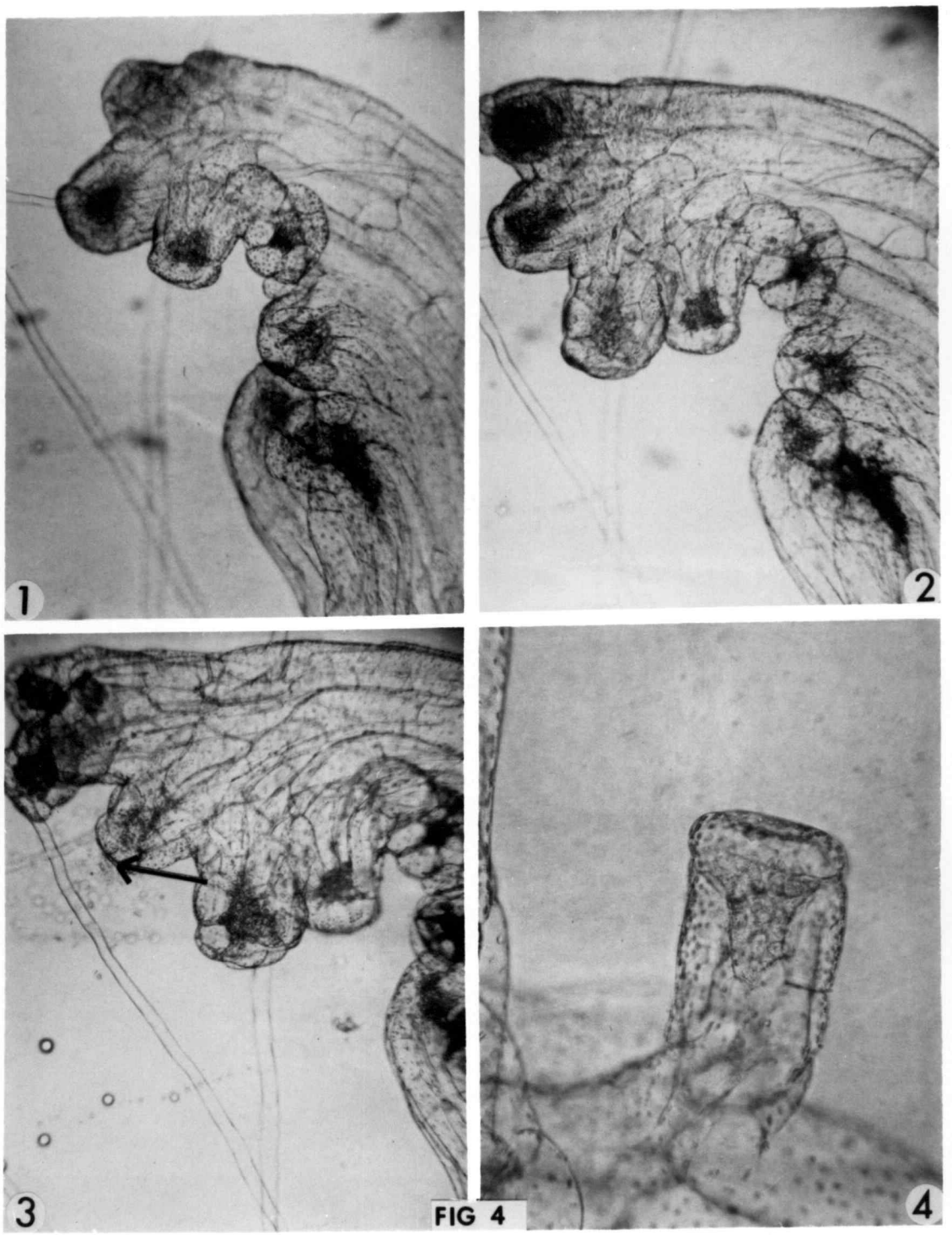


FIG 4

Figure 5-1. Release of sperm from E. telmateia antheridium. Thallus six and one half weeks old. 465X.

Figure 5-2. Immature archegonium on E. telmateia thallus five and one half weeks old. 115X.

Figure 5-3. Mature archegonium on E. telmateia thallus nine weeks old. 200X.

Figure 5-4. Equisetum telmateia gametophytes with young sporophytes in agar plate. Ten weeks old. 1.5X.

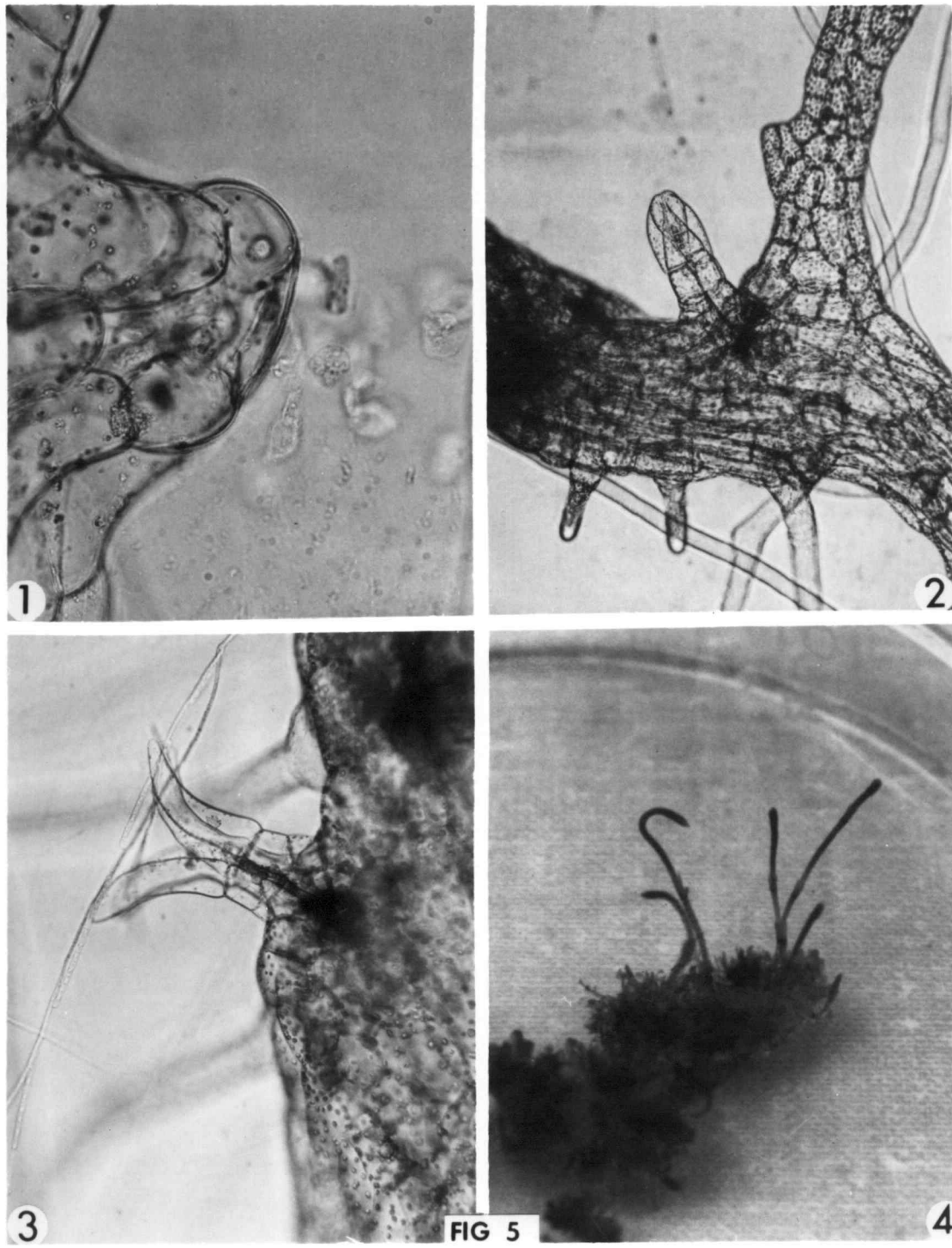


FIG 5

Figure 6. Relative size of chloroplasts in rhizoids and thallus cells of E. telmateia gametophytes. 395X.

Figure 6-1. Fifteen hours old.

Figure 6-2. Nineteen hours old.

Figure 6-3. Twenty-seven hours old.

Figure 6-4. Thirty-eight hours old.

Figure 6-5, 6. Three days old. Note size difference between thallus and rhizoid cells. Oil droplets are indicated in thallus cell.

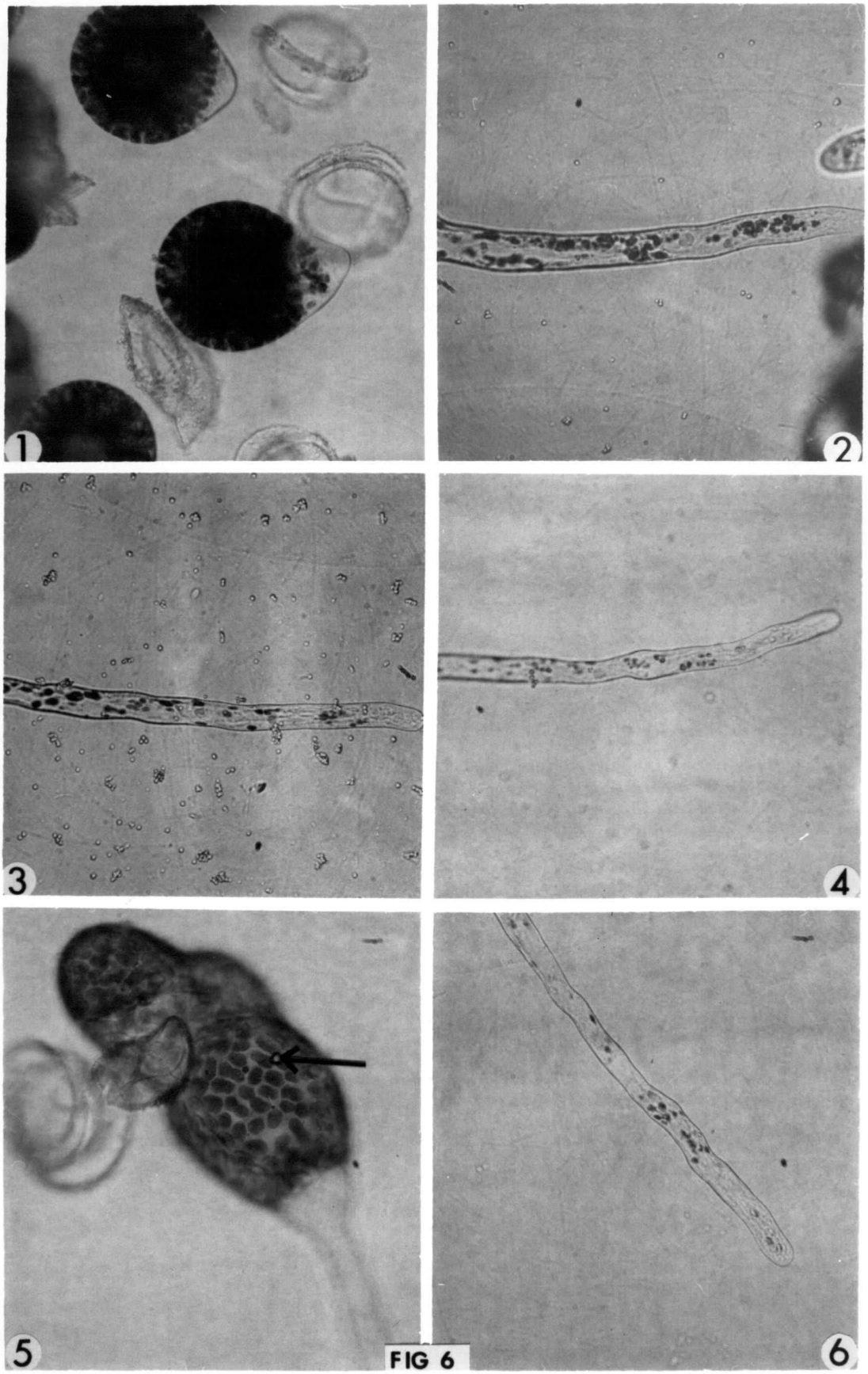


FIG 6

Figure 7. Division of chloroplasts in thallus cell of E. telmateia over a 29 hour period. From time-lapse movie sequence. Elapsed time between pictures 1.6 hours. 400X. Division of plastids a and b required the entire 29 hour period. The small plastid c divided rapidly and then fused.

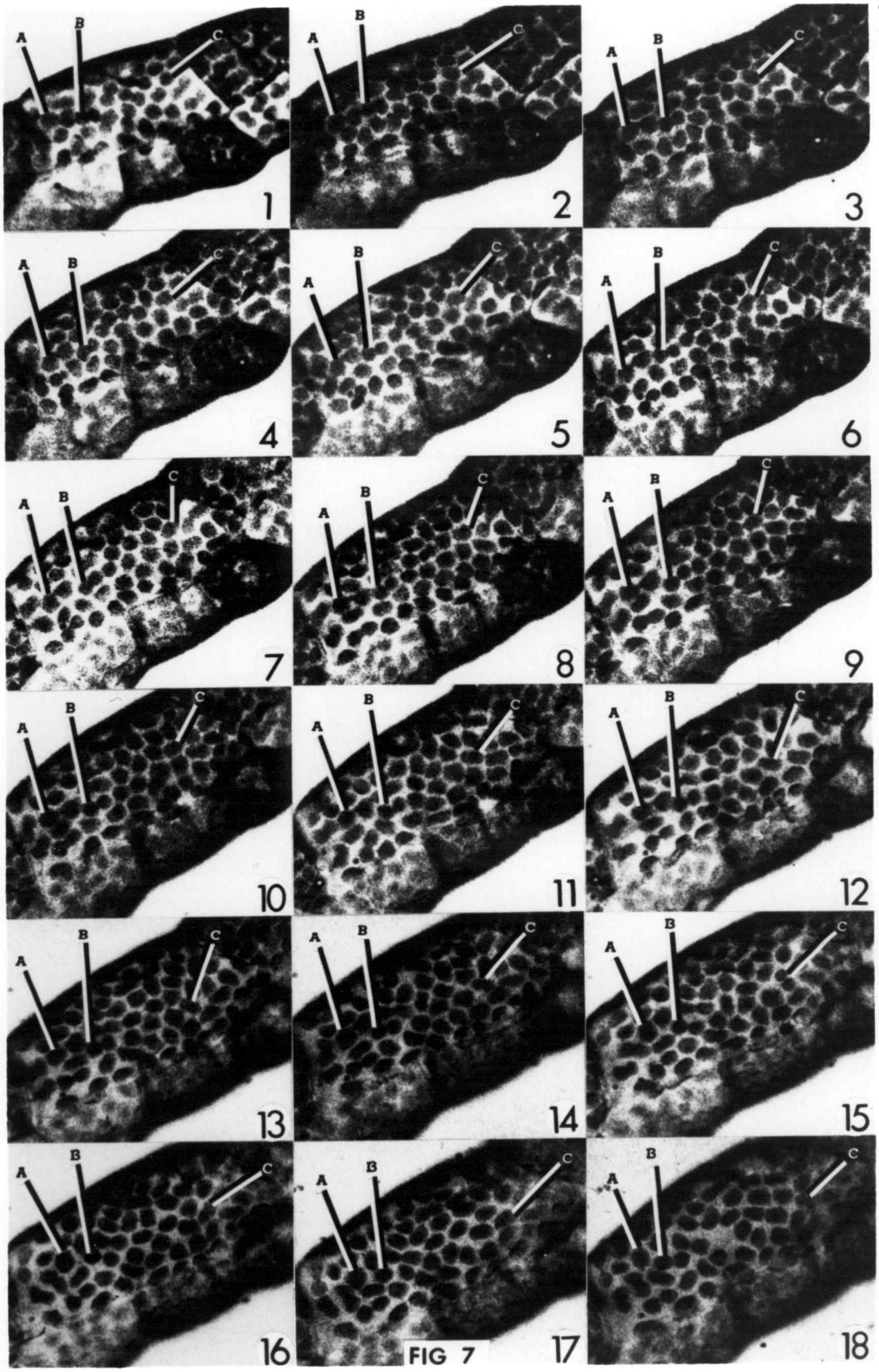


FIG 7

Figure 8-1. Comparison of chloroplast size between secondary rhizoid and thallus cells in E. telmateia gametophyte two weeks old. 200X.

Figure 8-2. Chloroplasts in elongated thallus cells of E. telmateia 15 days old. Giant plastids are indicated. 295X.

Figure 8-3. Portion of same thallus 16 days old. Giant plastid indicated. Mitochondria are seen as dark particles between the plastids. 395X.

Figure 8-4. The same cells 18 days old, and becoming senescent. 395X.

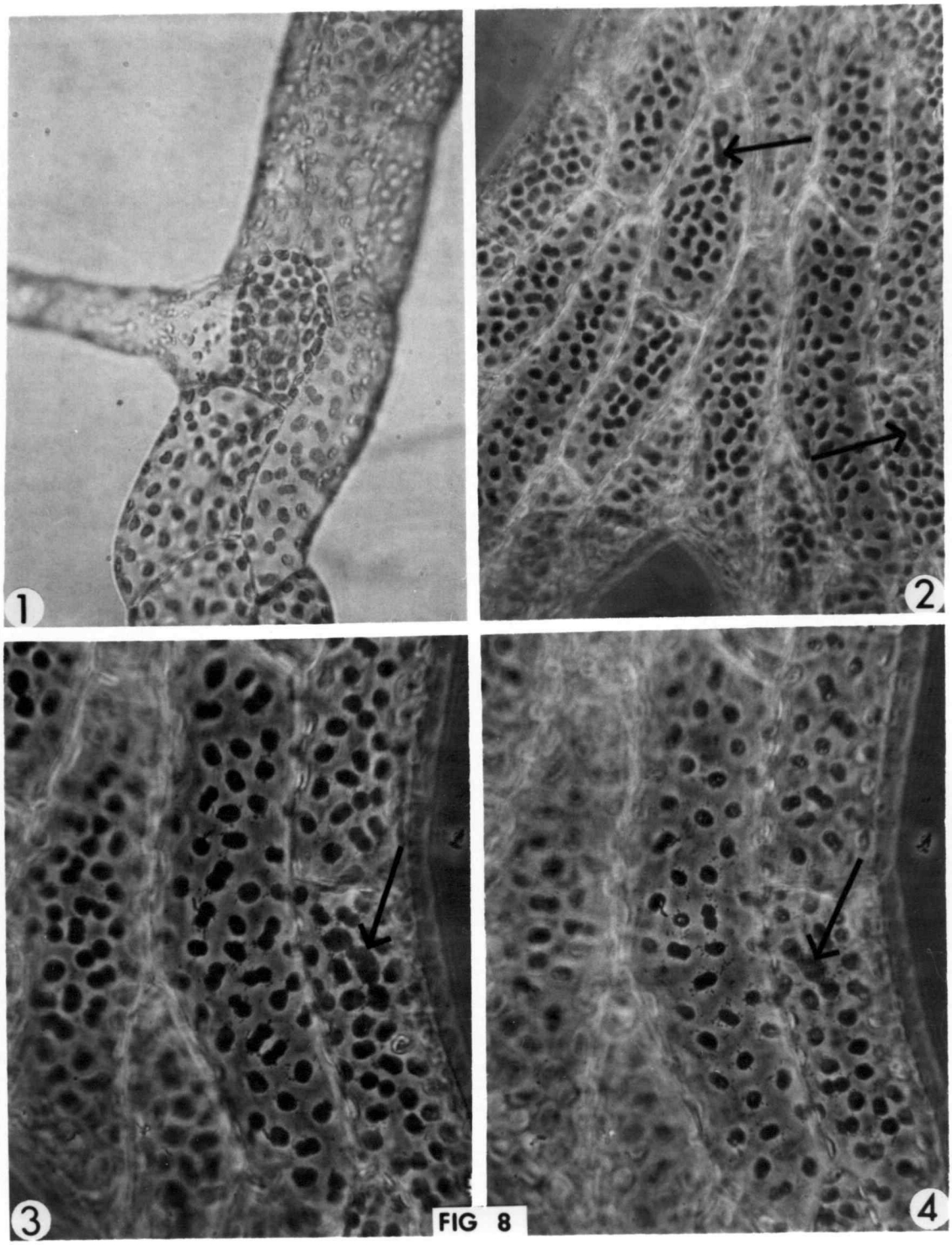


FIG 8

Figure 9-1. Decrease in chloroplast size in cells near developing antheridium. Thallus six weeks old. Note size decrease from cell 1 to 2 to 3. 465X.

Figure 9-2. Optical section lower within same thallus showing developing antheridium. 465X.

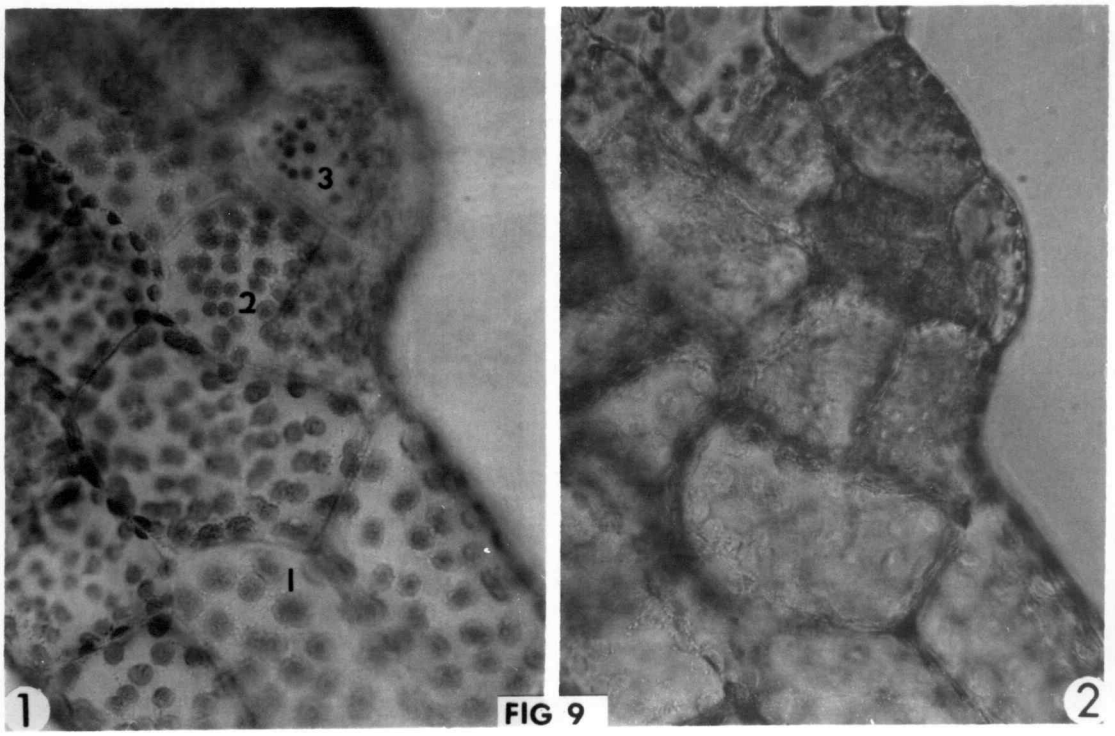


FIG 9

Figure 10. Chloroplast from thallus cell of E. telmateia gametophyte four days old. 64,000X. c.w. - cell wall; i.c. - intercellular connections; m. - plastid double bounding membrane; ER - endoplasmic reticulum sheath; gr. - granum; s. - starch; os. - osmiophillic granules.

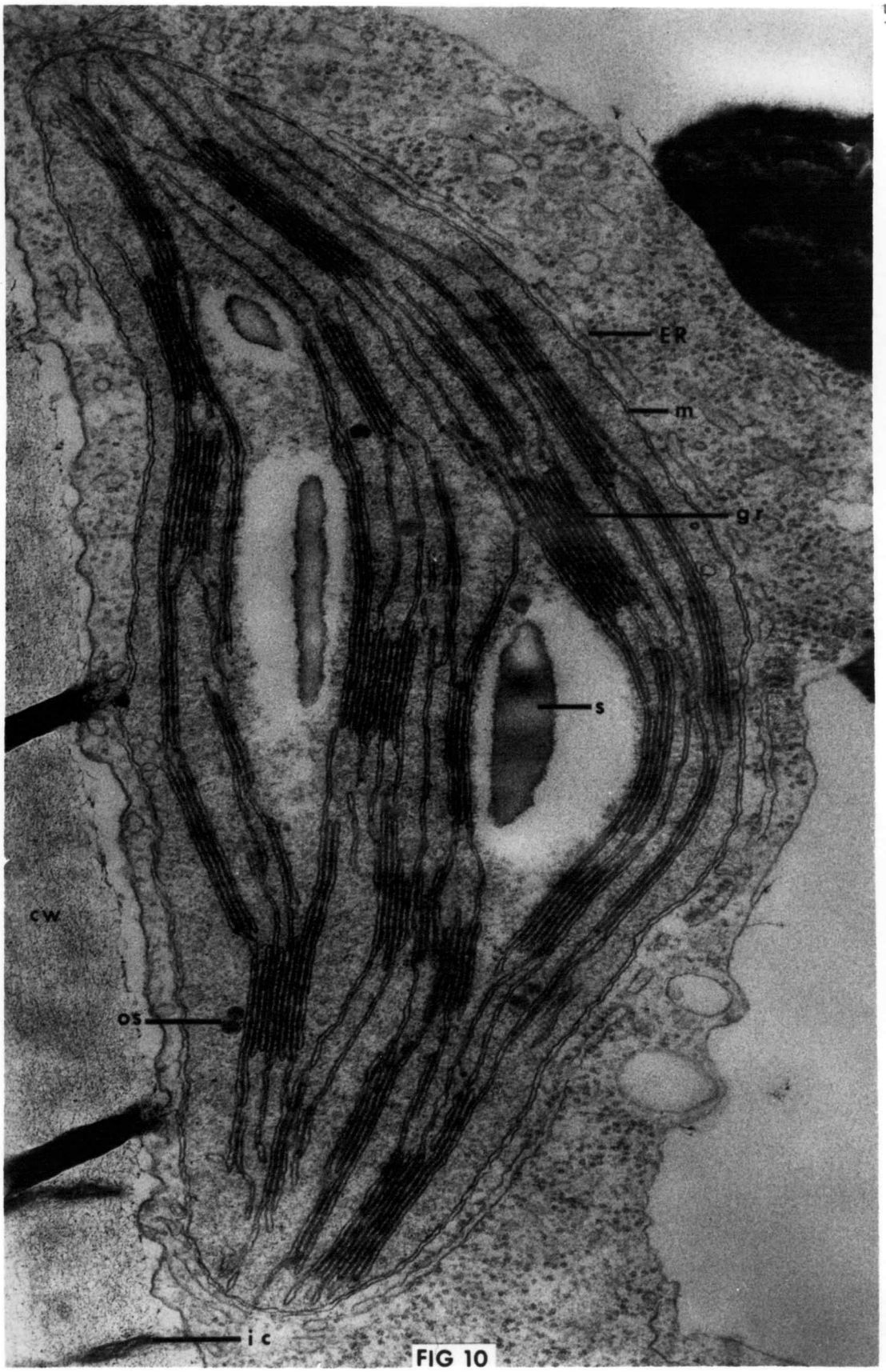


FIG 10

Figure 11-1. Recently germinated spore of E. telmateia. 295X.

Figure 11-2. Thallus cell cytoplasm from 22 hour old E. telmateia gametophyte. 4950X. Lipid globule indicated.

Figure 11-3. Chloroplasts from 22 hour old gametophyte thallus cell. 12, 375X.

Figure 11-4. Chloroplasts in 22 hour old rhizoid cell. 12, 375X.

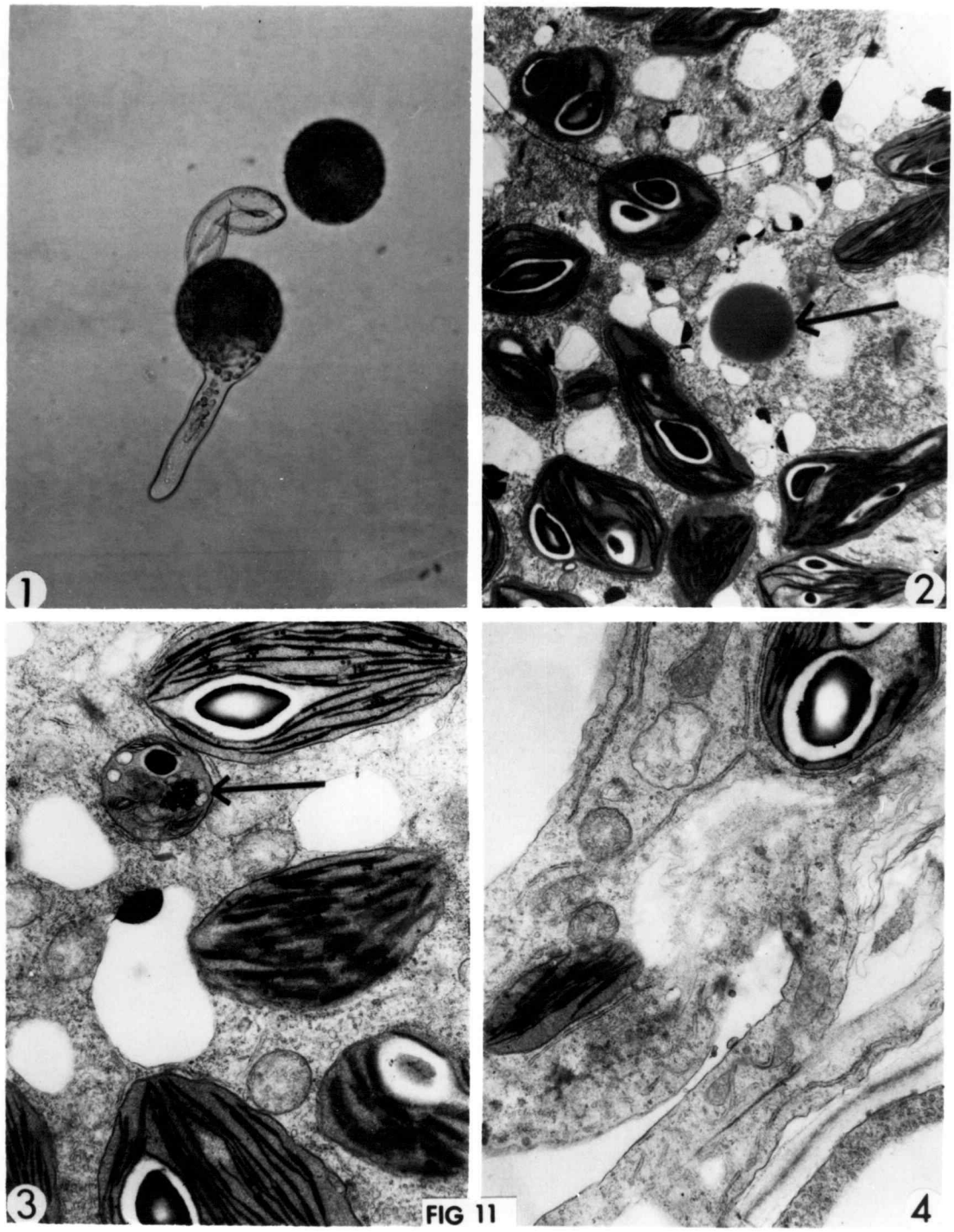


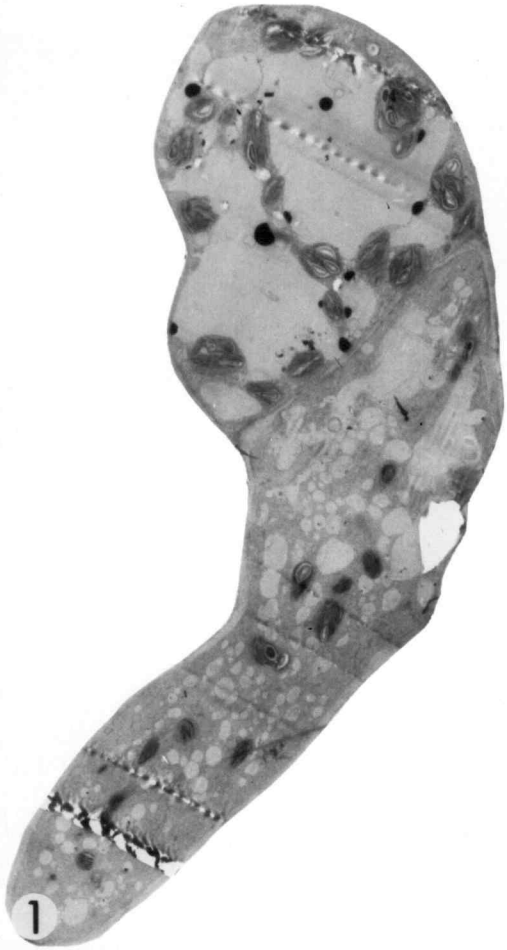
FIG 11

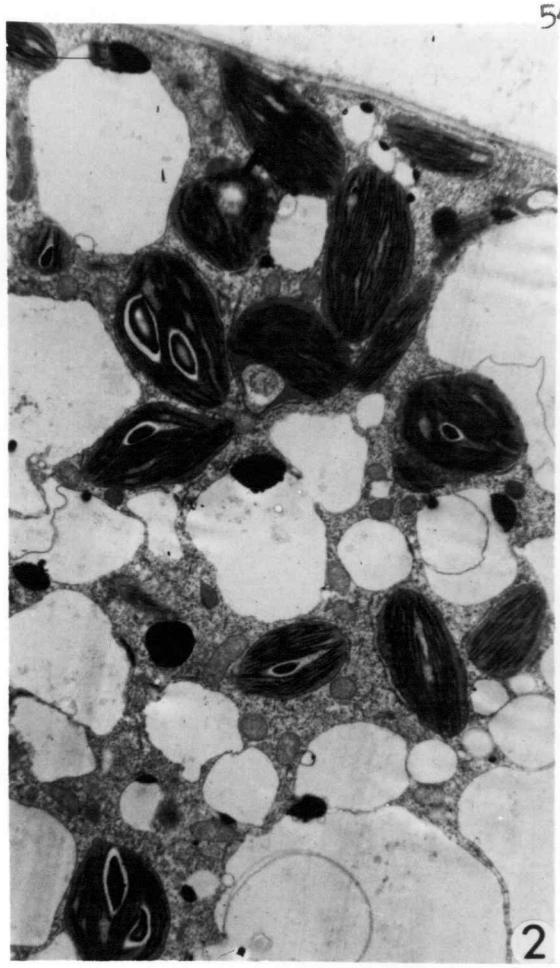
Figure 12-1. Twenty-six hour old E. telmateia gametophyte with thallus cell and rhizoid. 1800X.

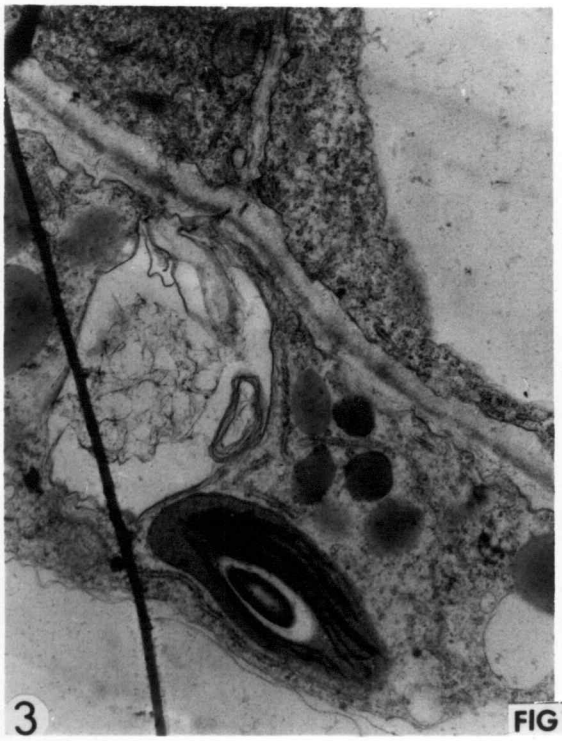
Figure 12-2. Twenty-six hour old thallus cell cytoplasm. 4950X.

Figure 12-3. Twenty-six hour old rhizoid cell separated by cell wall from two thallus cells on upper right. Note rhizoid plastid size. 15, 300X.

Figure 12-4. Twenty-six hour old thallus chloroplasts. Note plastid size as compared to those in rhizoid. 15, 300X.







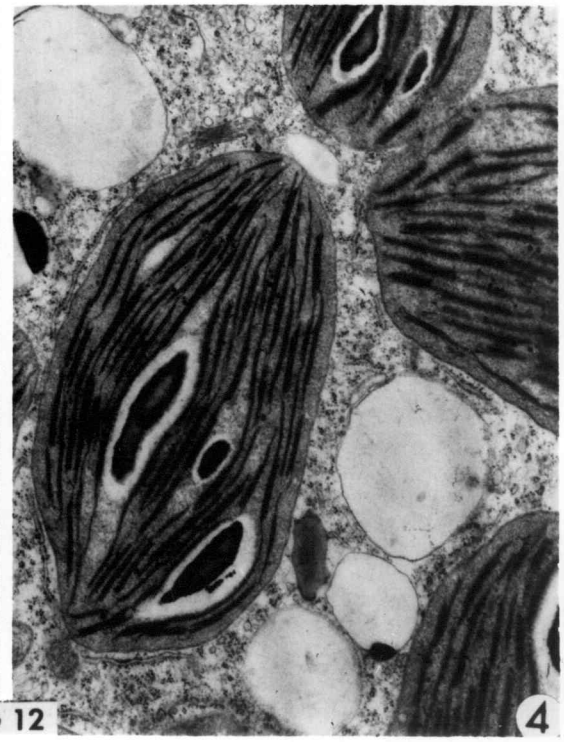


FIG 12

Figure 13-1. Thirty-nine hour old E. telmateia gametophytes with two thallus cells (t), and rhizoid (r). 1800X.

Figure 13-2. Thirty-nine hour old thallus cell (top), and rhizoid (bottom). Note difference in plastid size. 15,300X.

Figure 13-3. Cross section through 39 hour old rhizoid near tip showing dense cytoplasm and nucleus. 8950X.

Figure 13-4. Cross section through 39 hour old rhizoid near base showing large vacuole. 8950X.

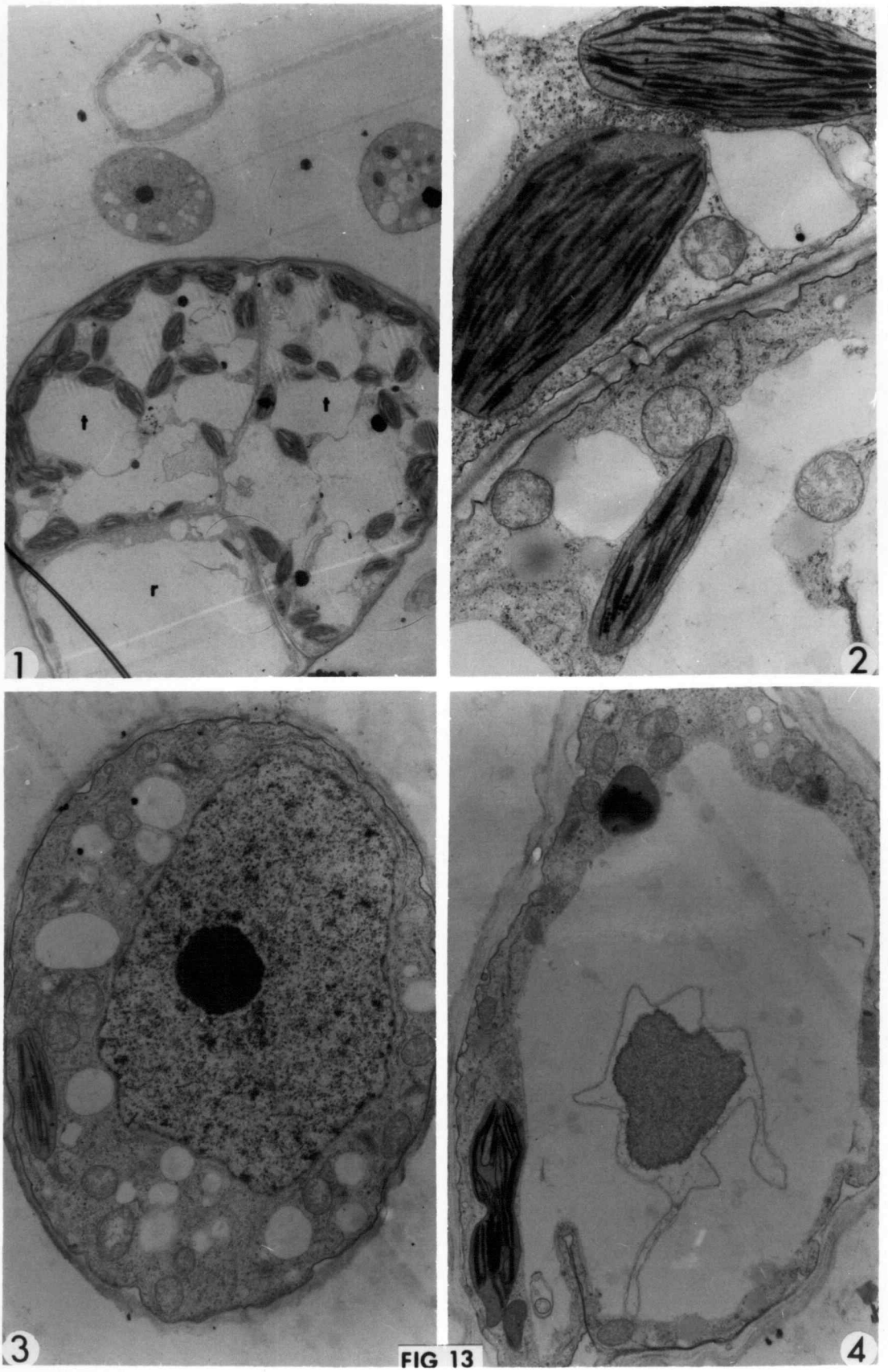


FIG 13

Figure 14-1. Four day old E. telmateia gametophyte. Note size difference between thallus cell plastid (t) and rhizoid plastids (r). 19,200X.

Figure 14-2. Four day old rhizoid cytoplasm with dictyosomes, ER, and degenerate plastid. 19,200X.

Figure 14-3. Eight day old live gametophytes of E. telmateia in microculture. 115X.

Figure 14-4. Electron microscope section showing tip cells of gametophyte shown in Figure 14-3. 660X.

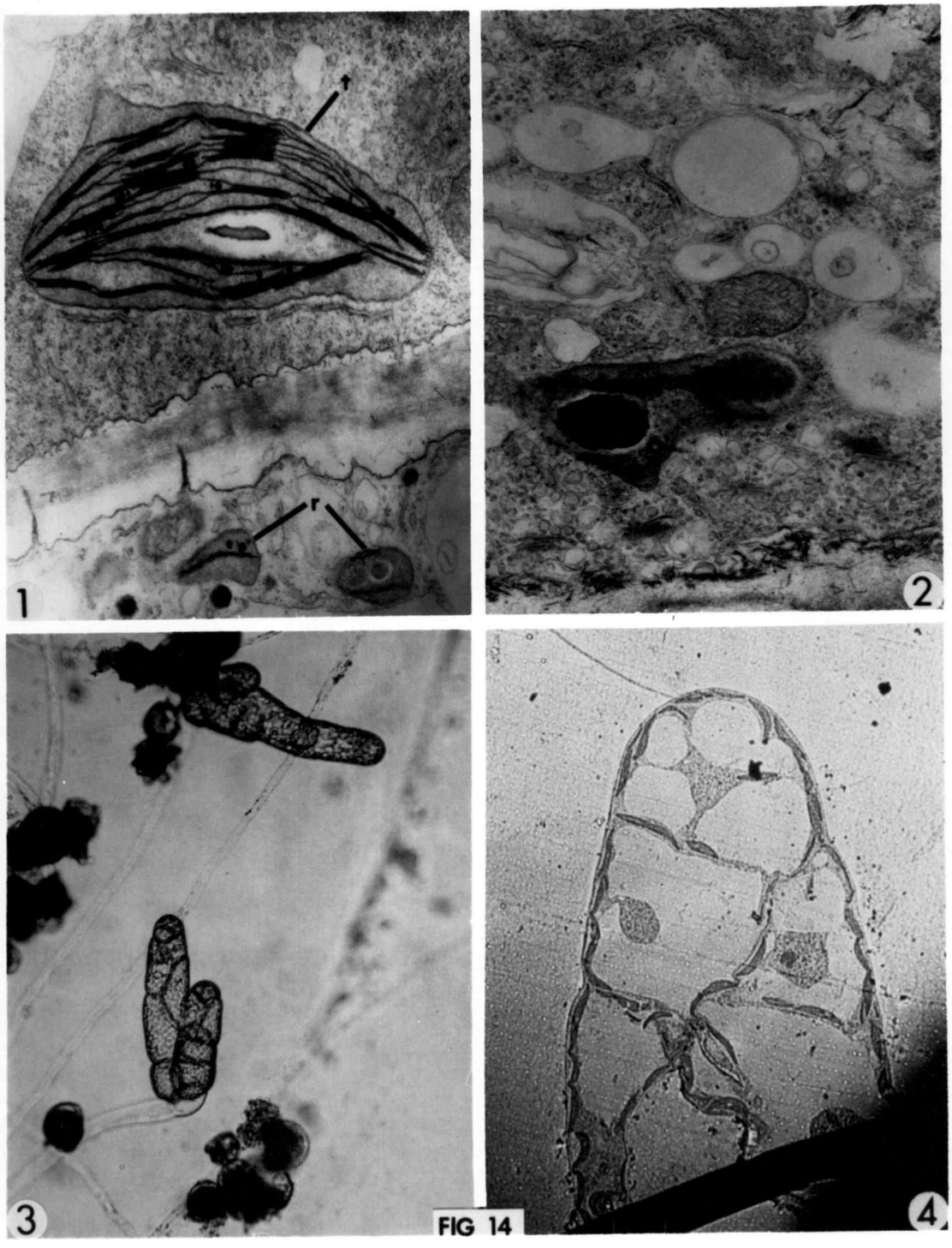


FIG 14

Figure 15-1. E. telmateia meristematic cell showing plastids oriented along strands supporting the nucleus. 4950X.

Figure 15-2, 3. Cell plate forming between daughter cells. c. - cell plate material; nuc. - daughter nucleus. Figure 15-2, 8,400X; Figure 15-3, 19,200X.

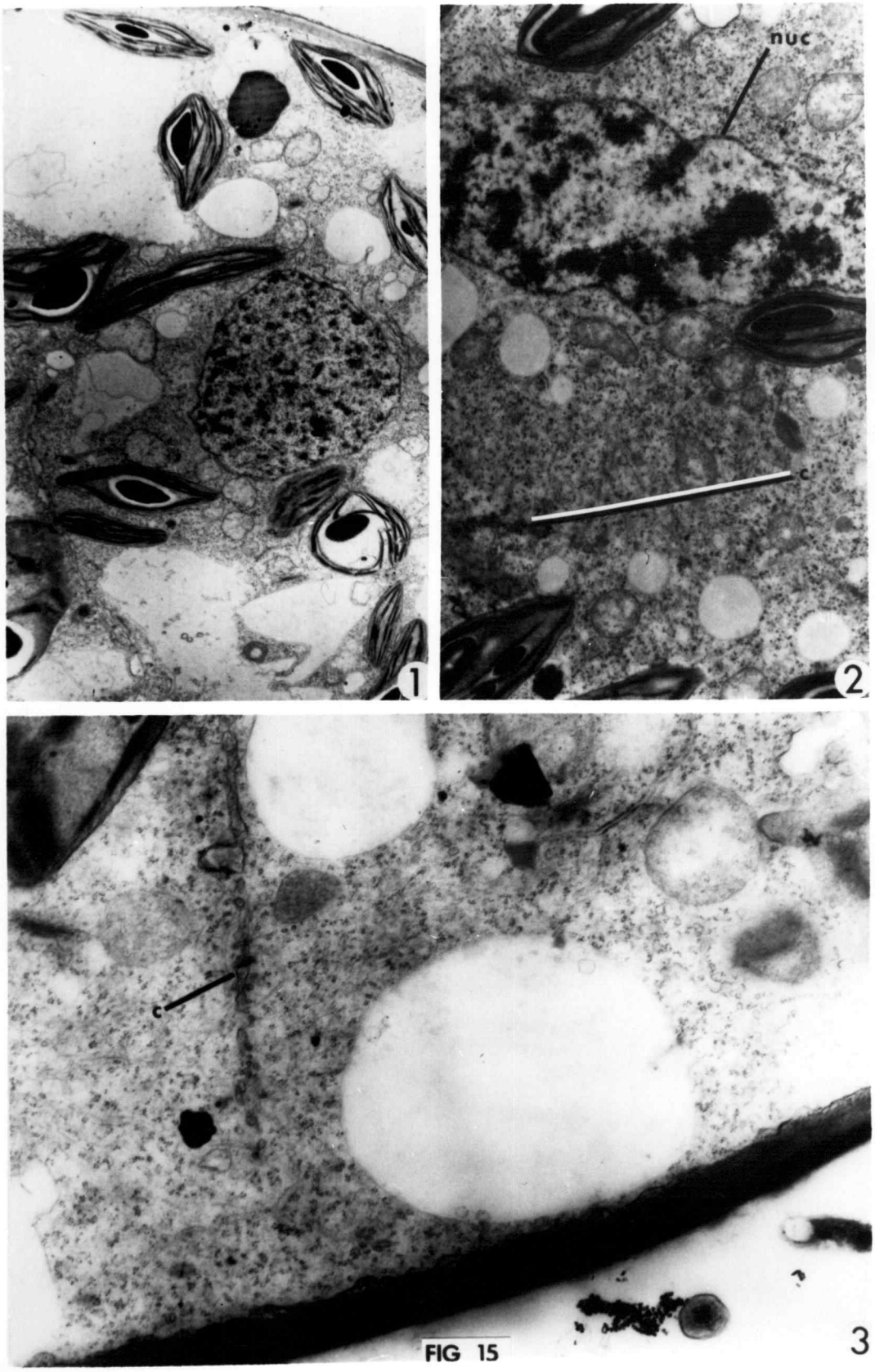


FIG 15

Figure 16. Two week old gametophyte of E. telmateia showing: r. - rhizoid; anth. - developing antheridium; m. - meristematic region. 594X.

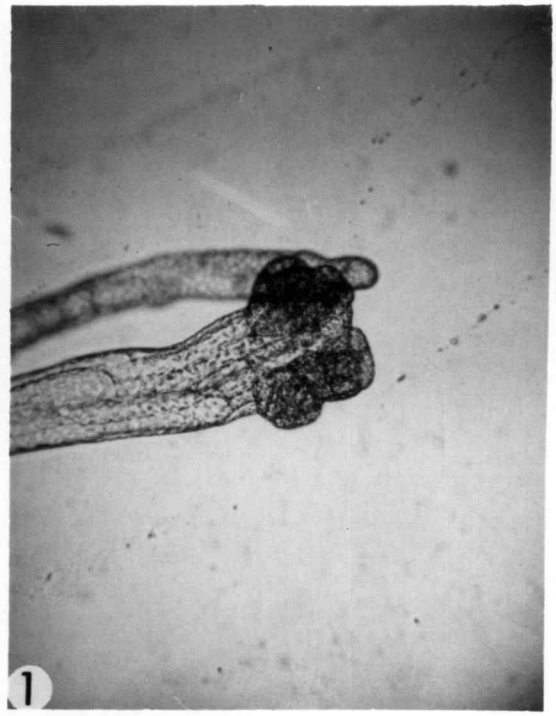
FIG 16

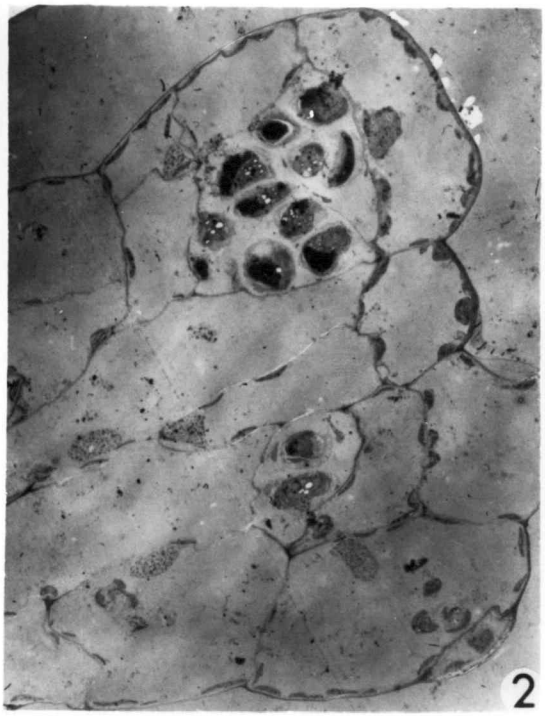
Figure 17-1. Developing antheridia on E. telmateia thallus 24 days old. 115X.

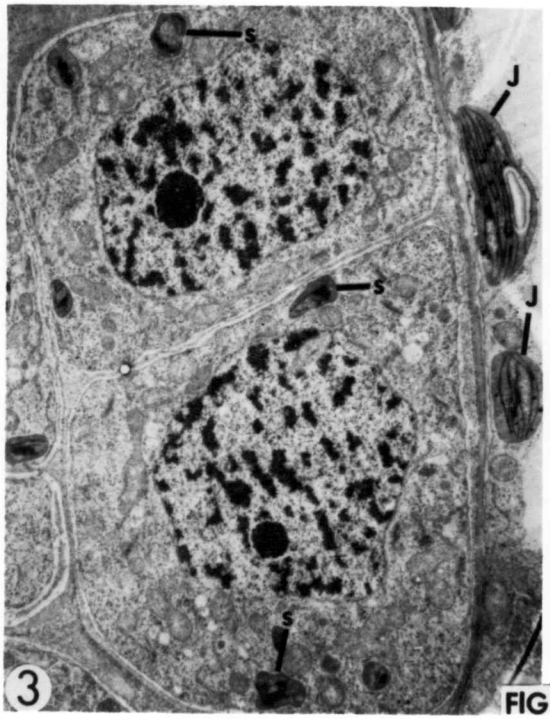
Figure 17-2. Electron microscope section through same antheridial head, showing developing sperm. 650X.

Figure 17-3. Antheridium with sperm mother cells. Note size difference between plastids in sperm mother cell (s) and in jacket cell (j). 6400X.

Figure 17-4. Single mature sperm: f. - flagellae; pl. - plastid. 19,200X.







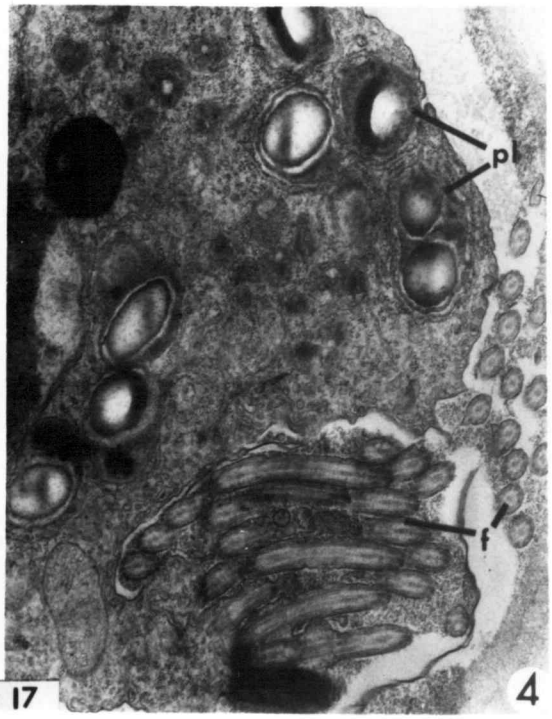
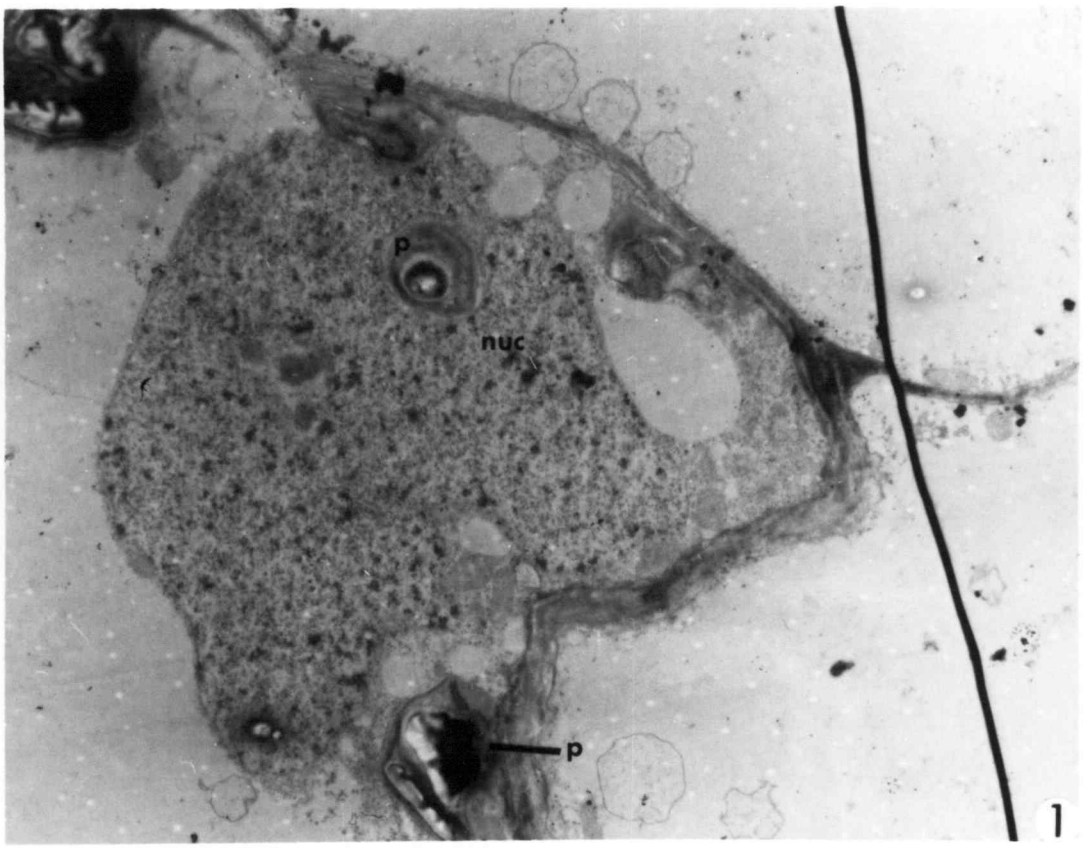


FIG 17

Figure 18- 1, 2. Lobed nucleus (nuc.) and plastids (p.) in senescent cell. Upper and lower pictures are sections through same cell at different levels. Figure 18-1, 6600X; Figure 18-2, 9200X.



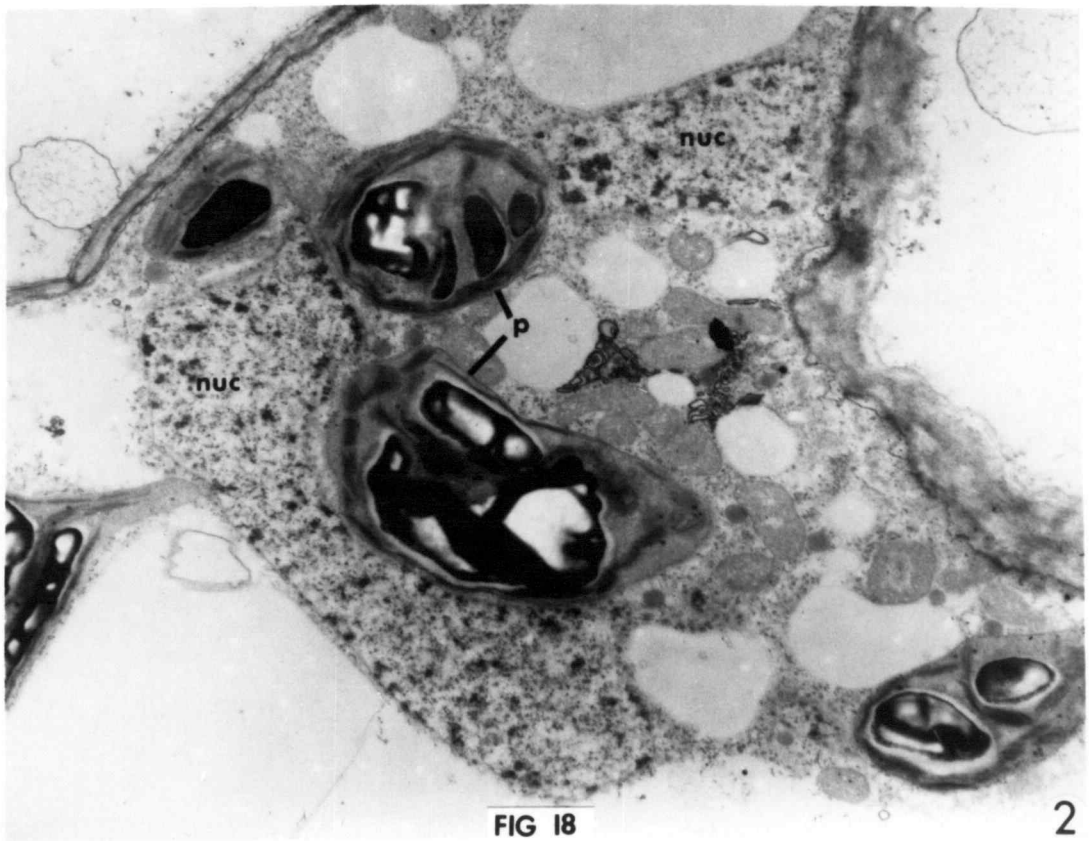
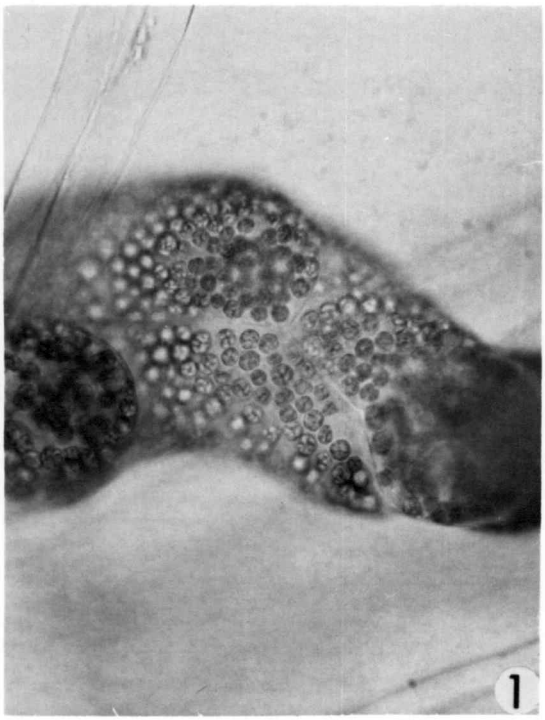


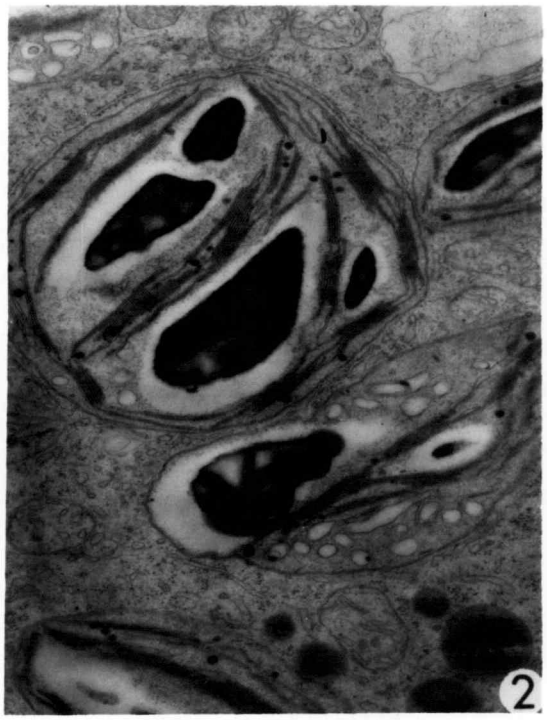
FIG 18

Figure 19-1. Degenerate plastids within live gametophyte one month old. Slide infested with bacteria. 395X.

Figure 19-2. Partially degenerate vacuolate plastids in four day old gametophyte from infested slide. 19, 200X.

Figure 19-3. Extremely degenerate plastid from same slide as Figure 19-1. 19, 200X.





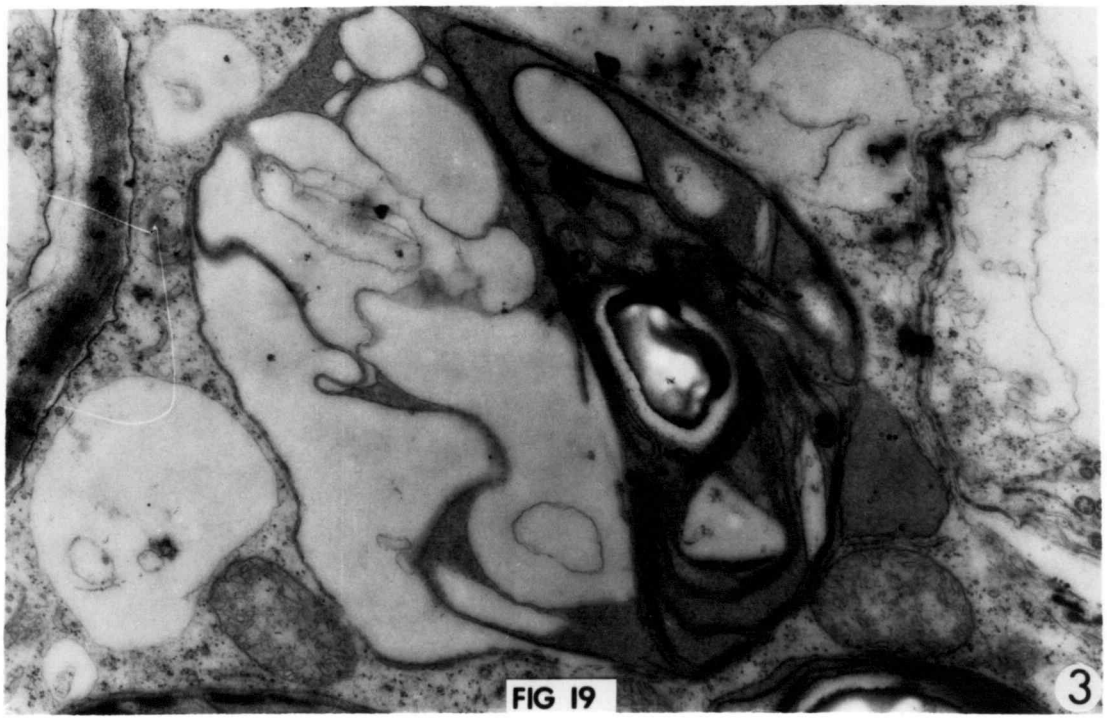
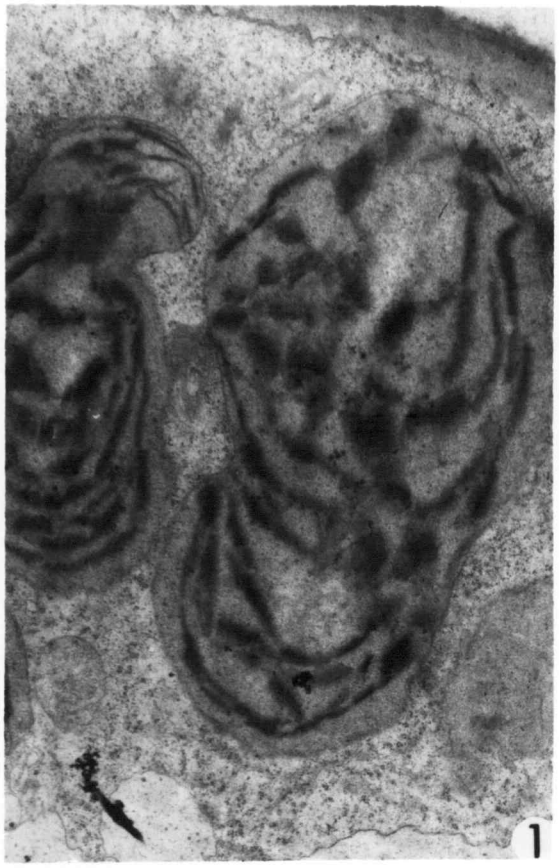
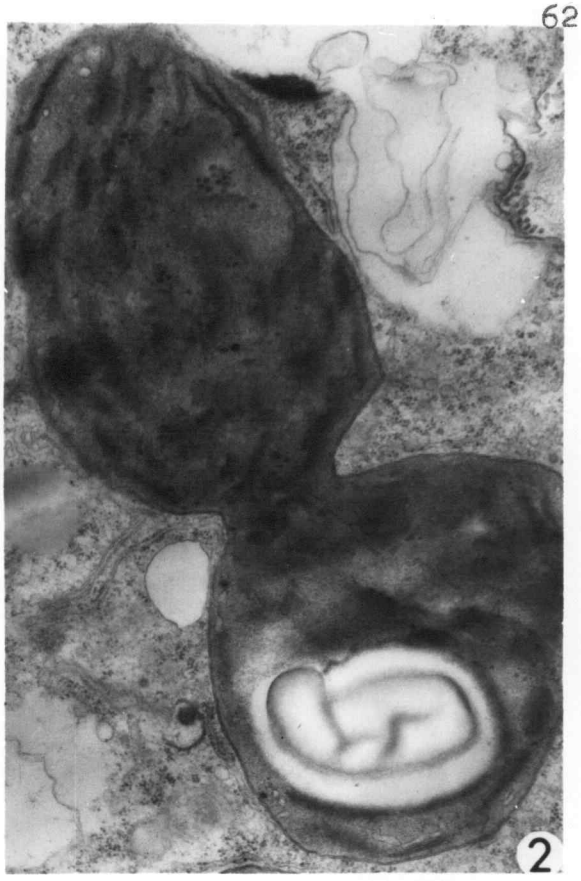


FIG 19

Figure 20. Dividing chloroplasts in E. telmateia thallus cells. Paradermal view showing various stages of constriction. Figure 20-1, 13,260X; Figure 20-2, 16,640X; Figure 20-3, 20,400X.





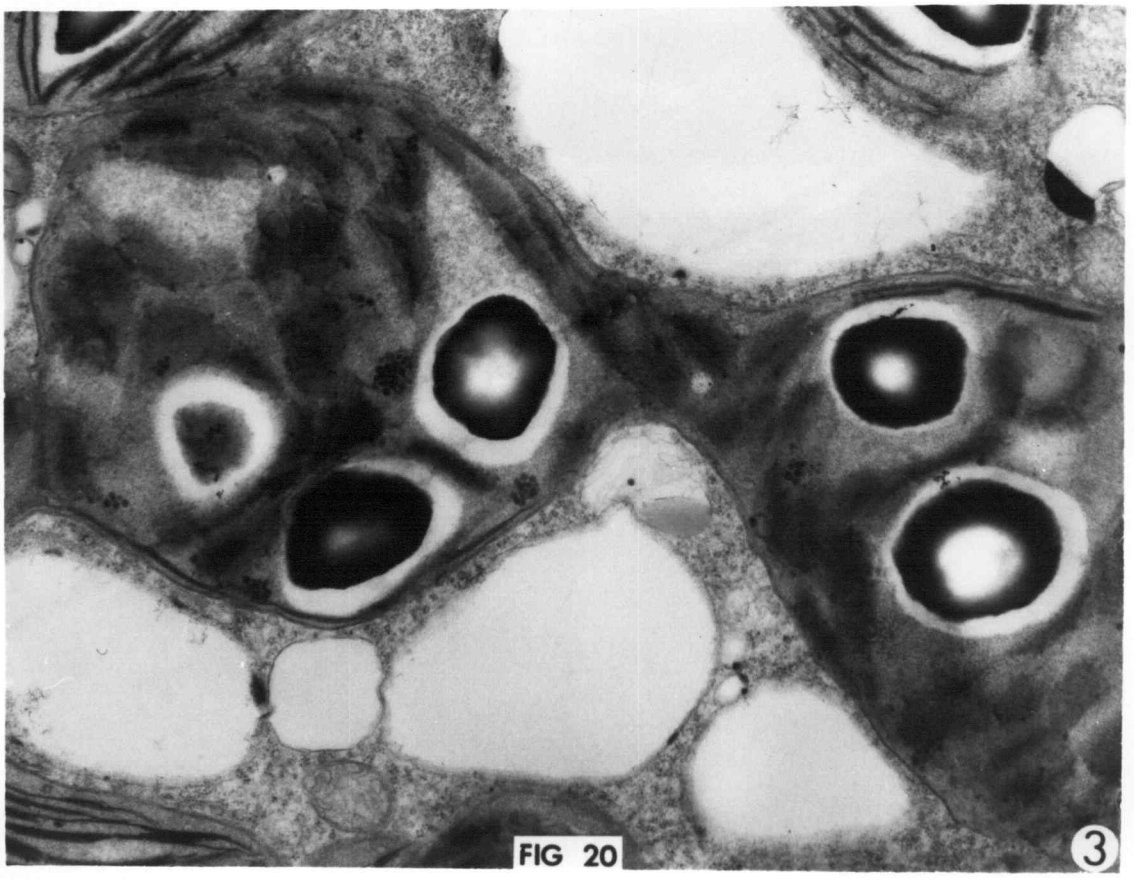
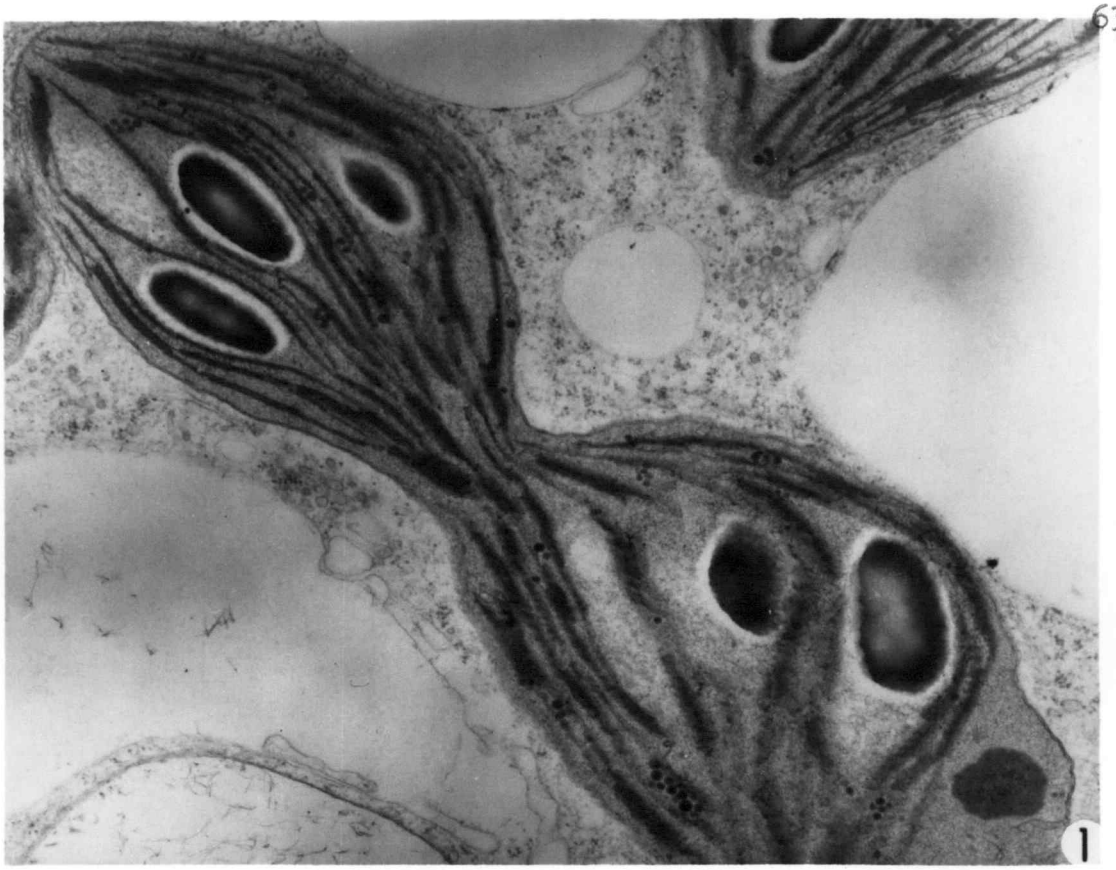


FIG 20

Figure 21. Dividing chloroplasts in E. telmateia thallus cells. Longitudinal view showing position of internal membranes during constriction. Figure 21-1, 25,600X; Figure 21-2, 16,500X.



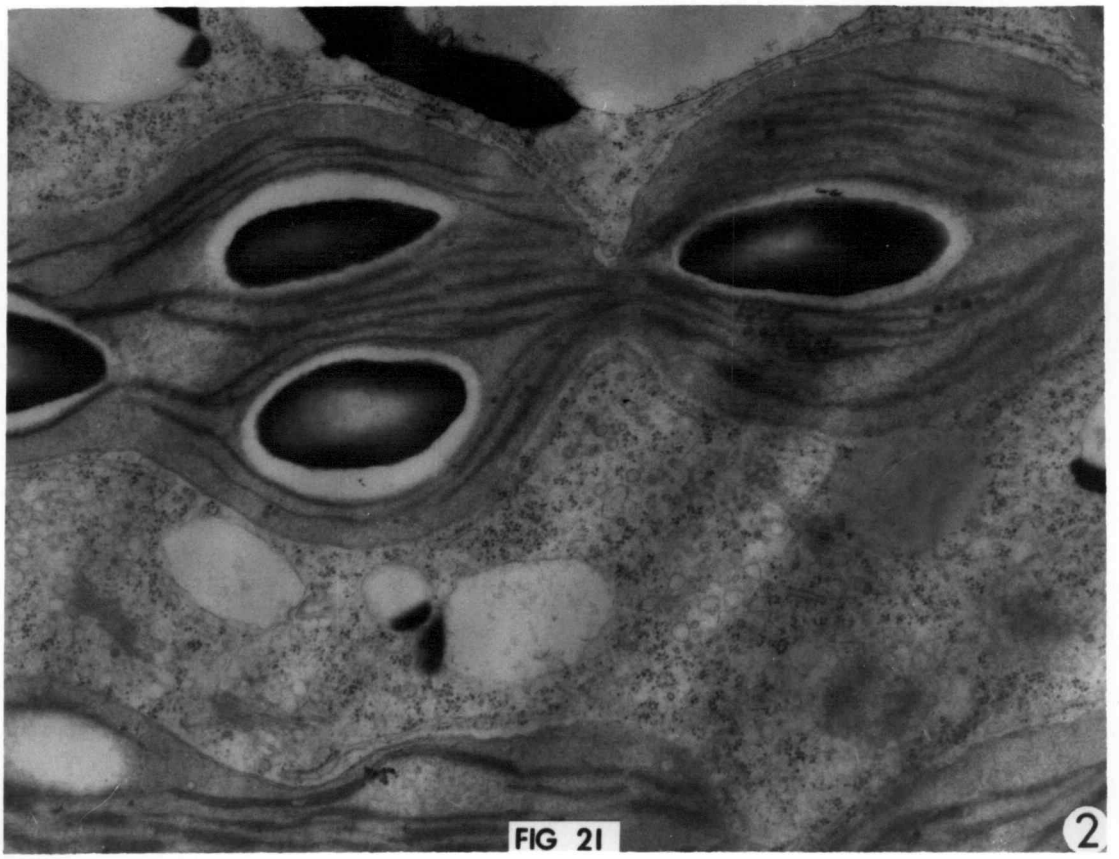
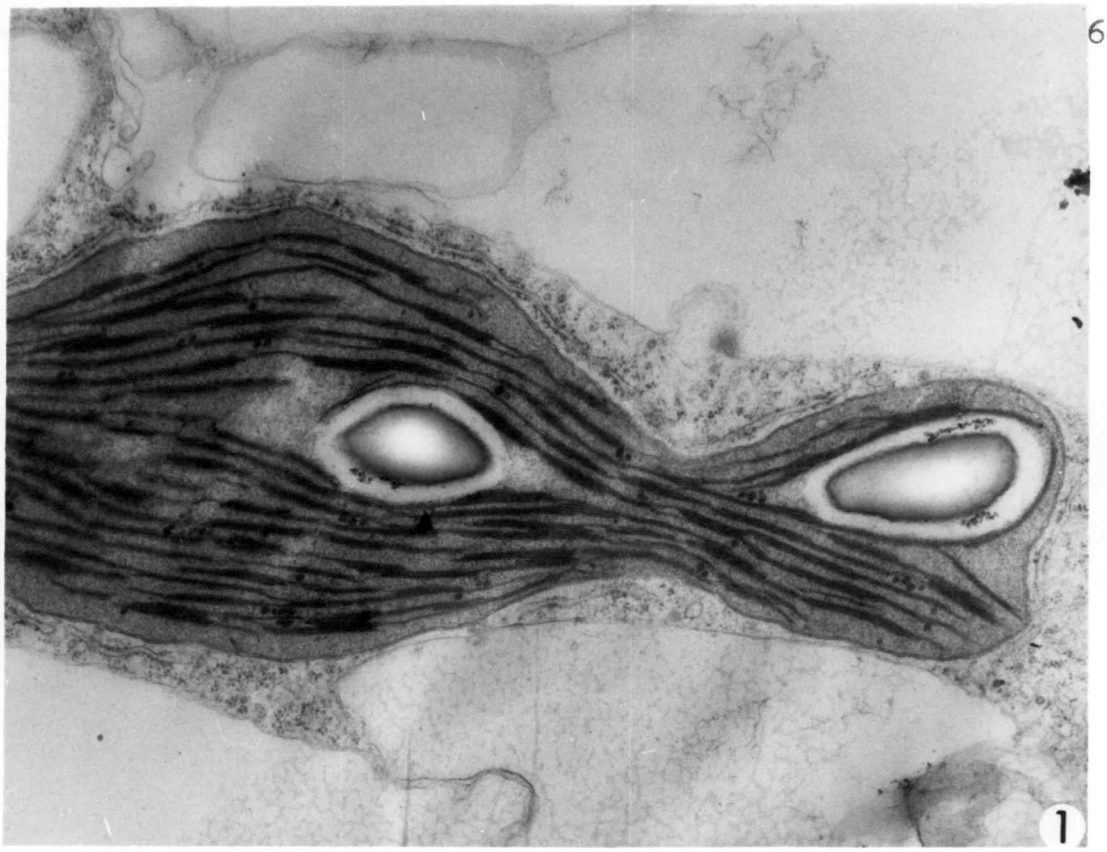


FIG 21

Figure 22. Dividing chloroplasts in E. telmateia thallus cells, showing position of internal membranes. Note granum turned at right angle in constriction of plastid in Figure 22-2. Both 25,600X.



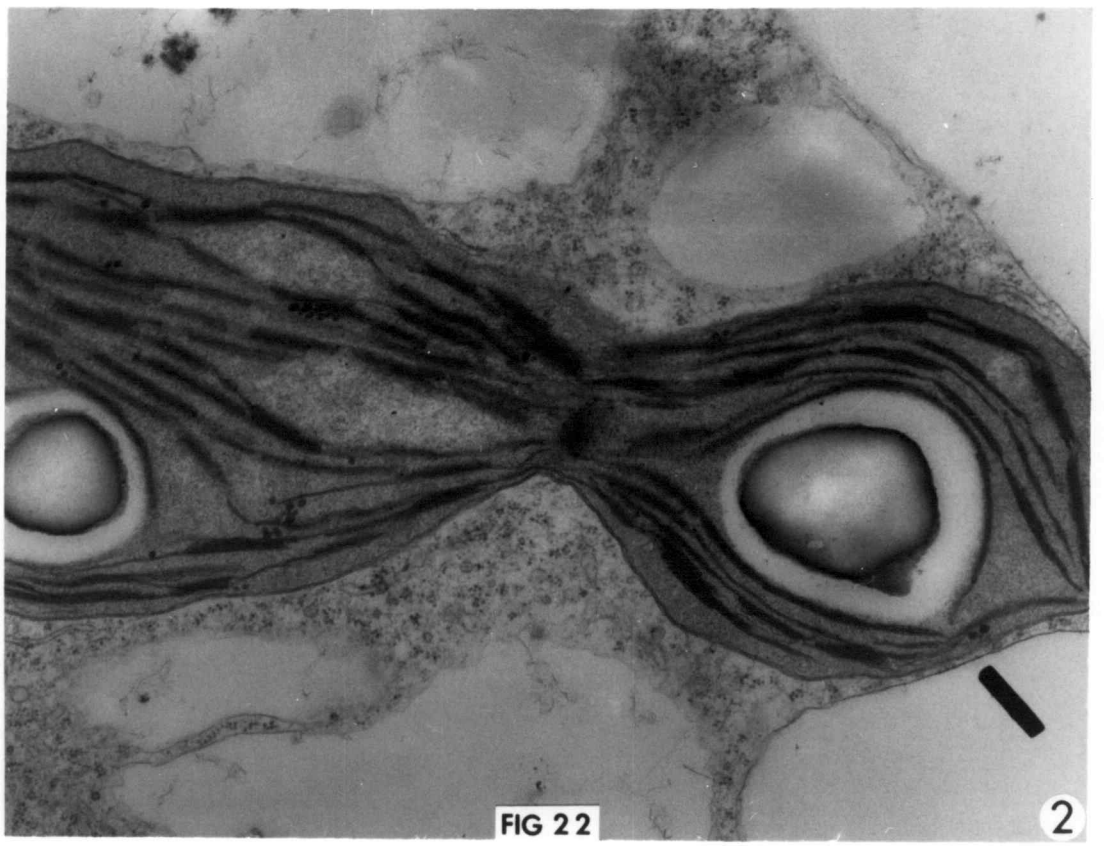


FIG 22

Figure 23. Chloroplasts in E. telmateia thallus meristematic cells, showing twisting of internal membranes. Right half of each plastid is embedded in parietal cytoplasm, left half is in cytoplasmic strand supporting nucleus. Figure 23-1, 32,000X; Figure 23-2, 25,500X.

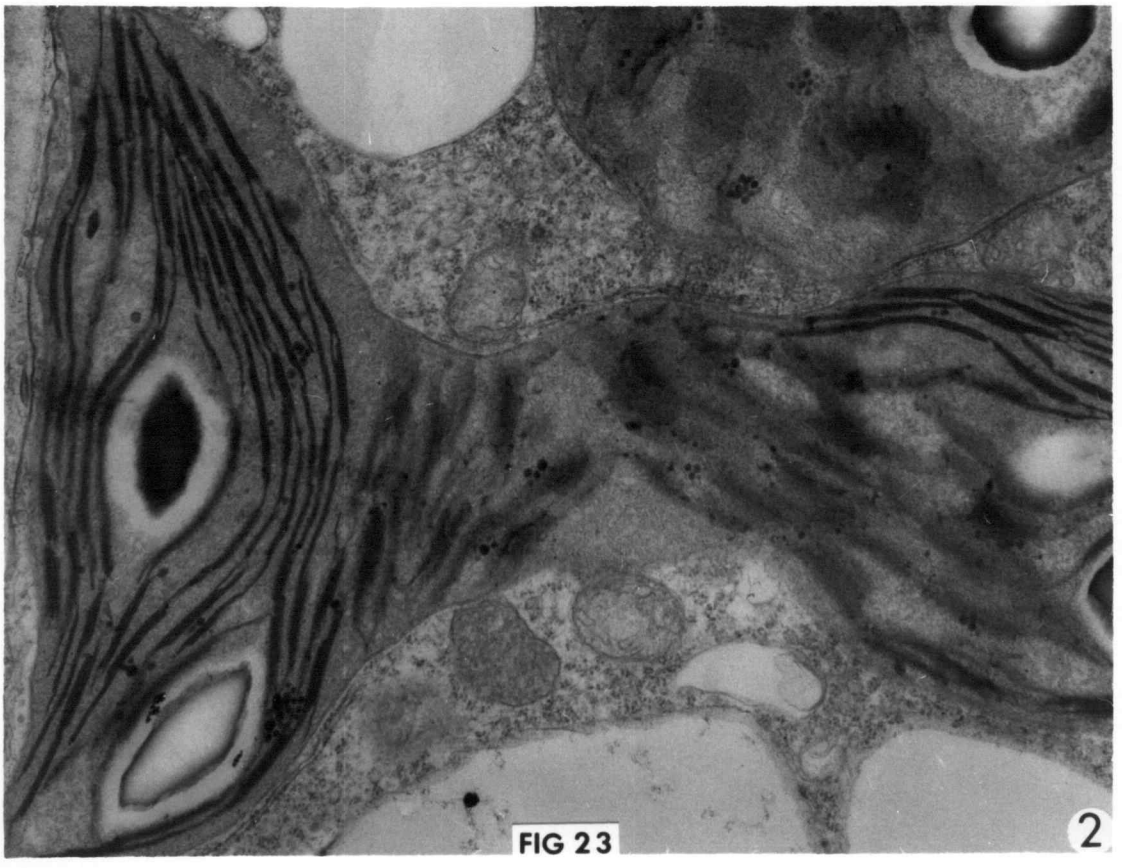


FIG 23

Figure 24-1. Dividing plastid in thallus cell showing possible means of bleb formation due to starch in area of constriction. 13,260X.

Figure 24-2. Chloroplast apparently dividing longitudinally in cell near antheridium. 16,640X.

Figure 24-3. Dividing plastid almost completely surrounded by ER sheath (ER). 13,200X.

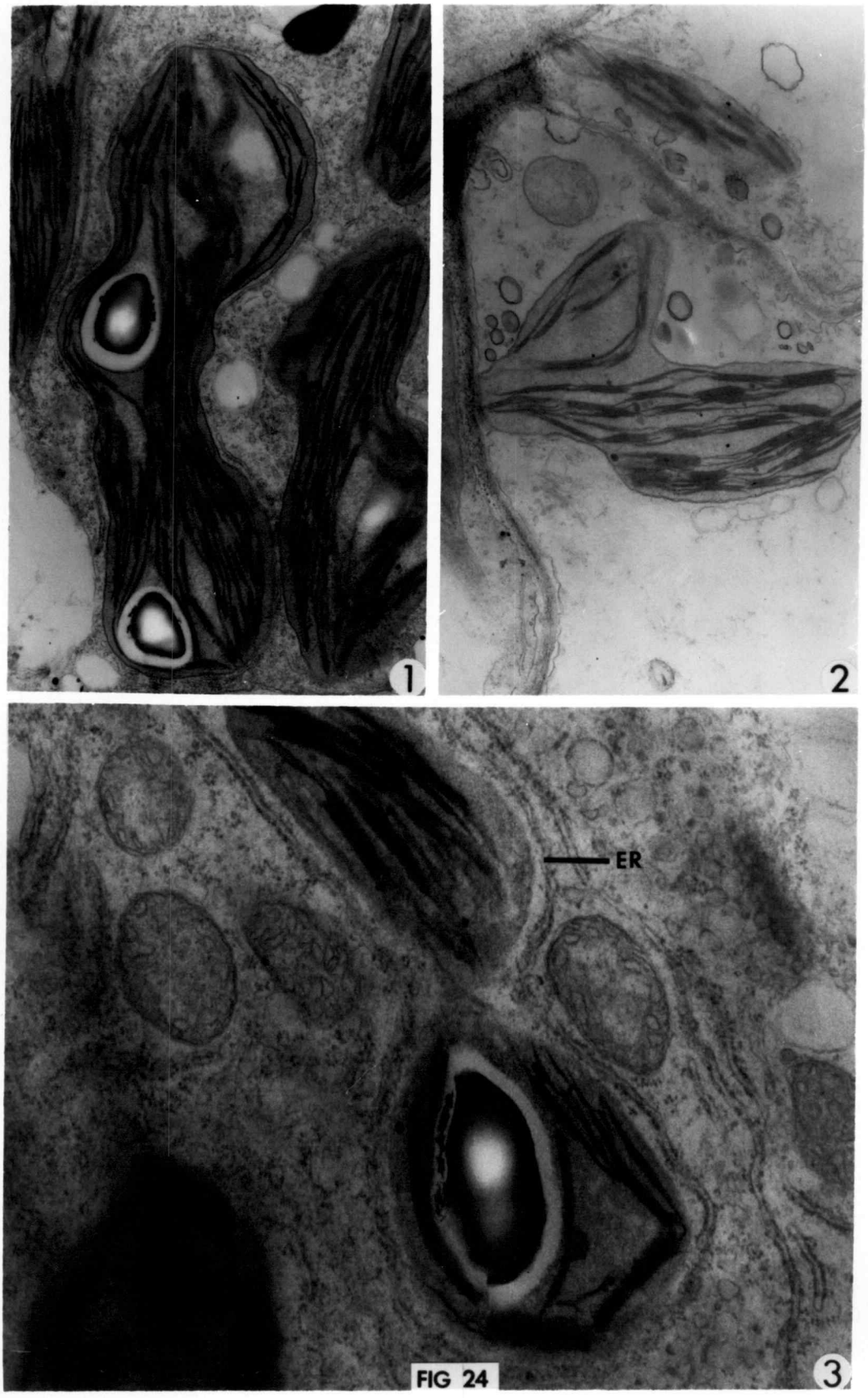
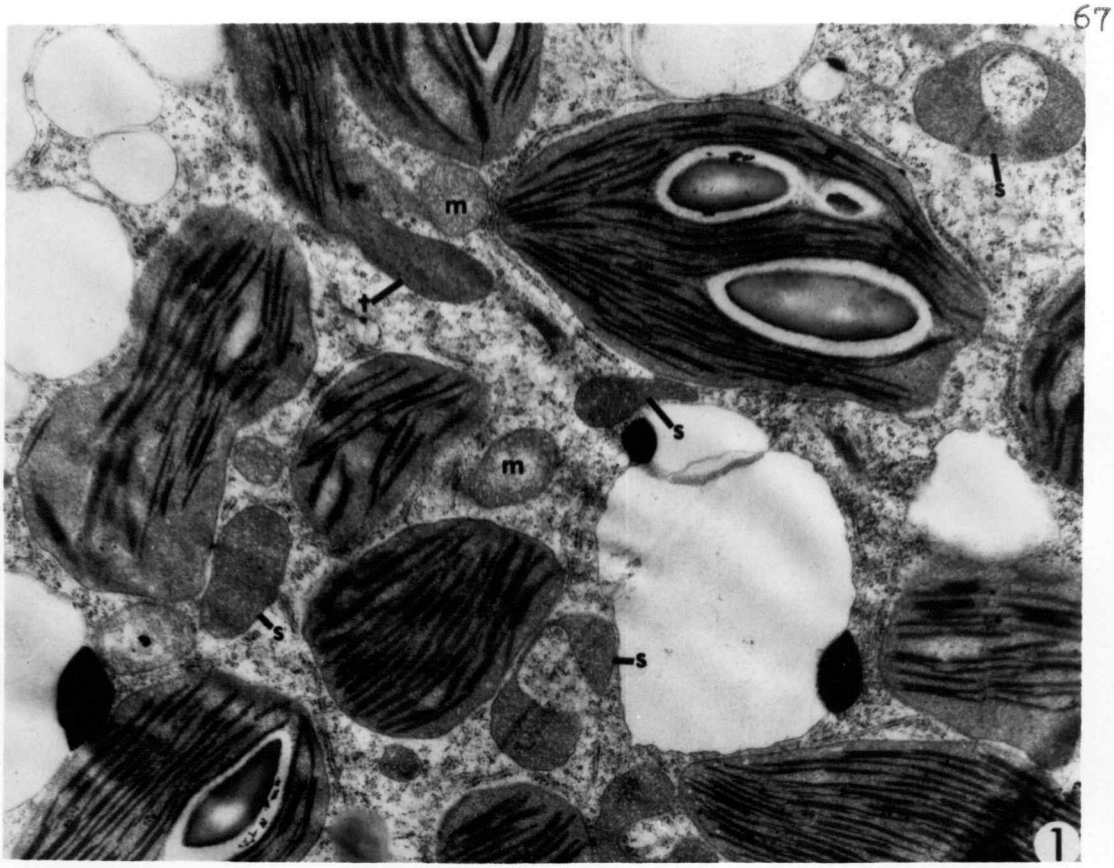


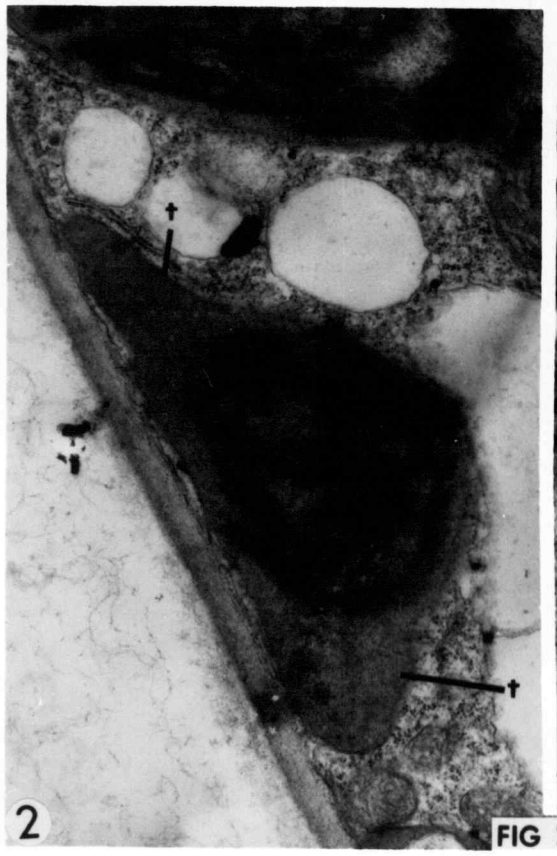
FIG 24

Figure 25-1. Equisetum telmateia thallus cell with: s. - stroma bodies; m. - mitochondria; and t. - chloroplast tail. 16,500X.

Figure 25-2. Chloroplast with tails (t). 19,200X.

Figure 25-3. Stroma body (s) near nucleus of same cell as Figure 25-2. 19,200X.





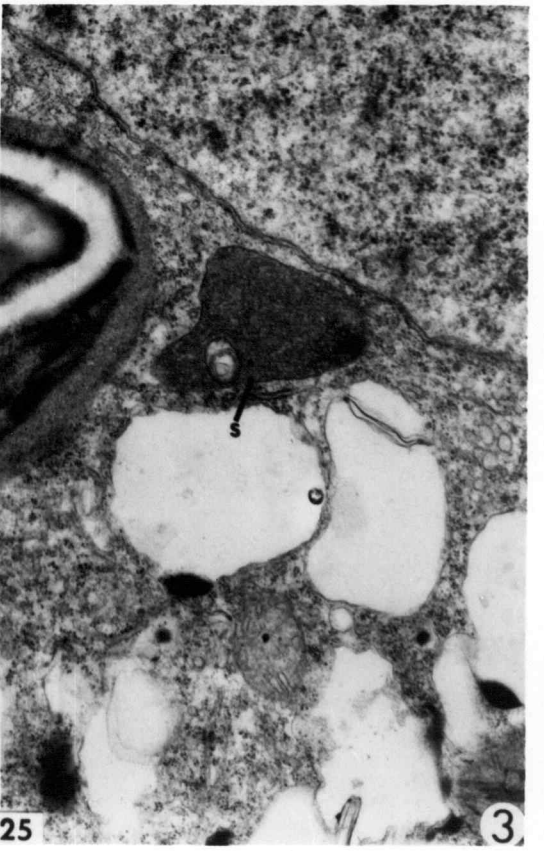


FIG 25