CYTO-MORPHOLOGICAL STUDY OF

PRASIOLA MERIDIONALIS

by

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ABSTRACT

A culture study of the morphology, cytology and life cycle of Prasiola meridionalis was conducted. The alga was grown successfully in Provasoli's culture medium (1958) at a temperature of 5^0C, and alternate periods of eight hours of light and sixteen hours of darkness. In culture, gametes are liberated only when mature reproductive or fruiting thalli are first illuminated for two hours with fluorescent light and then kept in the dark for several hours at temperatures of 3^0- 5^0C.

The results of the study have indicated that the life cycle presents a dimorphic alternation of generations. The gametophytic tissue and gametes represent the haploid generation, and the sporophytic tissue and zygote the diploid generation. Meiosis is intermediate in the life cycle, occurring in the adult sporophytic cells. Sixteen chromosomes were observed in the diploid cells, zygote, aplanospores and somatic cells.

Sexual reproduction is oogamous. The bi-flagellate micro-gamete is smaller than the macro-gamete which has no flagella. Asexual reproduction is accomplished by germination of aplanospores. Thalli which develop from germination of aplanospores and from zygotes appear the same morphologically.

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INTRODUCTION.

Although the genus Prasiola has been known since early in the 19th century, Prasiola meridionalis Setchell and Gardner was unknown until a century later. In 1920, Gardner (No. 3824) collected and Setchell and Gardner (1920) described the species from Neah Bay, Washington. They reported the presence of aplanospores in the thallus. Until the present investigation, no report has been made of number of chromosomes of this species, or its life cycle including its methods of reproduction. The present study was undertaken to report on its morphology, cytology, and life cycle in culture.

Agardh was the first to describe the genus Prasiola in 1822, but he was uncertain whether to call it a tribe or sub-tribe of Ulva. In 1838 Meneghini considered it as an independent genus, Prasiola. Several species of Prasiola have been described since then. In 1889 De Toni listed twelve species. Smith (1955) reported twenty species, indicating also that Prasiola is widely distributed, some species being marine ad others freshwater. Yatabe (1891) was the first to describe P. japonica Yatabe. Yabe's manuscript on the species was published posthumously by Ishii in 1932. P. japonica is the only species found in Japan. Eight species have been reported on the Pacific coast of North America. Four of these species, P. crispa (Lightfoot) Agardh, P. borealis Reed, P. delicata Setchell and Gardner, and P. meridionalis Setchell and Gardner are strictly marine; P.fluviatilis (Sommerfelt) Areschoug, P. mexicana Agardh, P. nevadensis Setchell and Gardner, are

found entirely in cold freshwater streams; and P. calophylla Meneghini, although terrestrial, is found in a marine locality.

P. stipitata Suhr was first described by Suhr in 1831, but it was not studied in detail. Drew distinguished the gametophytes among material collected in Bangor, North Wales in 1955. She verified the motility and fusion of the gametes in February, 1956, and in the following year all her observations and those of her co-worker Friedmann were jointly published under the title "Occurrence of Gametes in P. stipitata Suhr" (Drew and Friedmann, 1957). Friedmann (1959) undertook a more detailed investigation of the development and life history of the species. In 1960, Friedmann and Manton, with the aid of an electron microscope, studied extensively the gametes, fertilization and zygote development of the species.

Early workers studied the morphology and asexual reproduction of some species of Prasiola. Knebel (1936) studied the morphology of P. crispa. He also reported that Jessen, in his monograph of 1848, described the morphology of several species including P. stipitata. Gay (1888), Lagerheim (1892) and Wille (1901, 1906) discussed the vegetative reproduction of Prasiola by what they considered "gemmation of fronds and akinetes". In 1895 Borzi reported bi-flagellate zoospores in the life cycle of Prasiola. All the workers before 1931 agreed that Prasiola could propagate itself asexually.

Unlike the report of asexual reproduction, the one related to sexual reproduction was most controversial, being violently denied

and rejected by some investigators. Newton (1931) described the thallus and concluded that "sexual reproduction is unknown in Prasiola". Yabe (1932) remarked that both micro- and macro-gametangia are formed on the same frond. He reported the fusion of bi-flagellate micro- and macro-gametes. Several workers including Fritsch (1954) and Smith (1955) rejected the work of Yabe and all the previous reports. Uda's report of sexual reproduction in P. japonica in 1948 was confirmed and substantiated with photographs by Fujiyama in 1955.

MATERIALS AND METHODS.

Prasiola meridionalis used in this study was collected between the tides on rocks and boulders on the Pacific Coast at Friday Harbor, Washington on 26 July 1960; at Whiffin Spit, Vancouver Island, B.C. during the months of November and December, 1960; and at three localities in Stanley Park, Vancouver, from January through May, 1961. P. meridionalis is strictly marine, and during this study it has been found growing above sea water on the boulders encrusted by birds' feces. Prasiola thalli, found at low tide or above water, are matted together, holding moisture for sometime until the tide rises again. The thalli are dirty green (figs.1,2) and at the fruiting stage show alternate dark and light green patches at their apices.

The collected thalli were transferred to an ice-cooled container filled with sea water. In the laboratory they were cleaned carefully with a brush and transferred to dishes containing culture medium. They were kept at temperatures of 5^0 to 8^0C. The culture media used in this study were Lewin's artificial sea water (1955), sterilised sea water enriched with phosphates and nitrates, and Provasoli's medium (1958) modified by increasing the amounts of hormones so that the quantities are as follows for each 100 ml. of culture medium:

Adenine	3 milligrams
Gibberelin	10 micro-grams
Kinetin	10 micro-grams
Indole acetic acid	5 micro-grams

Provasoli's culture medium was brought to pH 8.0 or 8.2 . It gave the best results since the thalli cultured in the medium grew normally. Lewin's medium supported the growth of bacteria, and was not particularly conducive to the growth of P. meridionalis. Sterilised sea water enriched with nitrates and phosphates supported growth but the thalli became pale and unhealthy. A petri-dish was layered with filter paper soaked with the culture medium to keep the container moist. Slides were laid on the moist paper. The thalli of Prasiola meridionalis were laid separately and individually on the slides (fig. 1). Three times each day, drops of the culture medium were added to the thalli, the moist paper being changed twice a week. The petri-dish was covered and placed in a refrigerator at 5^0 - 8^0C. Illumination was provided by a fluorescent tube suspended over the culture plate in the refrigerator giving an illumination of eighty foot candles in alternate light (16 hours) and dark (8 hours) periods.

The method employed to aid the release of gametes is of particular interest. Friedmann (1960) noted that the gametes of Prasiola stipitata were released from the thallus if the fruiting thallus had been kept in the dark for at least sixteen hours. During this study, the fruiting thallus of P. meridionalis was laid on a slide and kept in the dark for 16 - 18 hours. A few drops of the culture medium were added twice a day. The gametes were released at temperatures varying from 4^0 to 5^0C. Thereafter, the slide was replaced by a new one thrice daily. Once the liberation of the gametes started in the dark, the presence or absence of light had no influence on release of gametes.

Some material was fixed in the field, and some at intervals after being cultured in the laboratory. Several fixatives were satisfactory if the material was thoroughly washed after fixing. During this study, formalin-acetic-alcohol (100 c.c. ethanol, 7 c.c. glacial acetic, and 40 c.c. 40% formaldehyde) gave the best results. Acetic-alcohol (1:3) was successful, particularly with the Feulgen staining technique. The fixed thalli were kept in tightly closed bottles in an oven at a temperature of 75^{0}C. for about twelve days; the fixative was changed every four hours until the thalli were thoroughly bleached. The thalli were passed through 70% - 50% - 30% alcohol and washed thoroughly in distilled water. They were sometimes stored in 70% alcohol. Although Karpechenko's fixative (Darlington and La Cour,1960) bleached the thalli as well, it required much longer washing, and the result obtained did not justify the time expended. Moreover, this fixative is chemically unstable after a short time even when stored in the dark. Therefore, it is prepared in two parts, and mixed just before it is used.

Staining was carried out on the whole pieces of thallus, gametophytic tissue and the zygote. The best results were obtained with propiono-carmine (Darlington and La Cour, 1960) which stained the chromosomes more conspicuously than the pyrenoids. One or two drops of propiono-carmine were added to the thallus on a clean slide, and after forty seconds, a cover slip was applied. The slide was warmed gently over a spirit flame. Pressure was applied to spread the cells before the cover

slip was sealed with a paraffin-gum mastic compound. The staining of the thallus with modified aceto-carmine (Friedmann 1959, 1960) required a much longer time but yielded good results, particularly after the stained thallus had been cleared in 45% acetic acid. Feulgen stain (Storvel, 1954; La Cour, 1960) was found appropriate only to verify the presence of nuclear material, since the chromosomes were not clearly defined.

During this study, motion picture of the gametes, syngamy and the zygote were taken. Photomicrographs were also taken using 35 mm. Kodak high contrast copy and Kodak D-11 developer for maximum contrast. Prints were made on Kodak bromide F3, single weight paper and developed in cobrol. Camera lucida drawings were made directly from microscope.

OBSERVATIONS.

Stages of the life cycle of Prasiola meridionalis observed in culture during this study include the adult thallus, gametes, syngamy, zygote, the developing stages of the zygote and aplanospores. The general type of life cycle (fig. 3) observed in P. meridionalis is diplo-haploidic since a macroscopic diploid sporophyte alternates with a microscopic haploid gametophyte, the two generations being morphologically dissimilar. A more detailed life cycle of P. meridionalis is presented in Fig. 4. The micro- and macro-gametes are released from the apex of the thallus. While the ellipsoidal micro-gamete is bi-flagellate the large, spherical macro-gamete has no flagella. Syngamy occurs shortly after the gametes are released. The zygote undergoes mitosis and an obovate thallus forms following further cell division. The cells at the apex of the thallus undergo meiosis to produce haploid cells. These cells then divide mitotically and produce cells which therafter constitute the gametophytic tissue. The female and male gametes are produced by cells in patches of the gametophytic tissue. The union of the male and female gametes starts the life cycle over again.

An asexual part of the life cycle is observed simultaneously as an accessory method of reproduction. The mature cells from the side and center of the thallus enlarge and differentiate. Each one divides mitotically to form four or six daughter cells, called aplanospores, all contained within the mother cell wall or a common coat (fig. 28). On release, each aplanospore divides mitotically in a manner similar to that of the zygote to form an adult thallus.

The adult thallus of P. meridionalis consisting of both sporophyte and gametophyte is obovate in shape and dirty green in colour (figs. 1,2). It is 7 - 17 mm. in length (figs. 2,5) and its width varies from the rhizoid to the apex, being about 2 mm. near the base, 8 mm. at the middle of the thallus, and 12 mm. or more at the apex. The rhizoid, the somatic tissue and the gametophytic tissue are easily distinguishable. The short colourless rhizoid is held fast to the substratum by a mucilaginous substance secreted by the basal cells (figs. 6, 7). The somatic tissue (figs. 8, 9) is uniformly dark green in colour. Its cells are of uniform size and are arranged in an irregular fashion (figs. 7, 9). The gametophytic tissue at the apex, or top third of the thallus, can be identified macroscopically. It contains irregularly shaped patches of dark green cells alternating with the groups of very light green cells, presenting a mosaic pattern (figs.8,10). The dark green cells produce female gametes, and the light green cells, the male gametes. Unlike the rhizoidal and somatic tissues, this tissue is poly-stromatic being four or six cells deep. The patches of the dark green cells are usually two to four cells deep, and those of light green cells are usually four to six cells deep.

The cells of the diploid part of the thallus are morphologically alike but differ in size. Each cell is uninucleate. The nucleus is located near the edge of the cytoplasmic membrane, next to the green chromatophore and on the same plane with it, but not so easily seen (fig.11). The conspicuous chromatophore is axile in position with finger-like

projections radiating towards the periphery of the cell. Within the center of the green chromatophore is a single pyrenoid (figs. 11, 13). The chromatophore in the basal cell is inconspicuous and the cells are light green or colourless. The basal cells are the largest in the thallus, each being 6 - 8 microns long. This variation in the size of the cells is more conspicuous in the growing thallus. The diploid somatic cells of the mature thallus average 8.3 microns in diameter.

Two types of cell division, mitosis and meiosis, were observed among the cells of the thallus. Mitosis occurs in the rhizoidal and somatic tissue of the thallus. Meiosis, however, is restricted to the top or uppermost cells of the sporophytic tissue at the apex of the thallus.

At the beginning of mitotic division, the somatic cells enlarge considerably from 8.3 microns in diameter to 22 microns in length and 7.3 microns in width. The nucleus becomes granular with a single nucleolus in the center. The chromosomes are seen clearly at the early mitotic prophase but they are not individually distinguishable at late prophase or metaphase. Sixteen chromosomes were counted at early prophase and at metaphase (fig. 12). Generally only eight or nine chromosomes were seen in one focal plane. The chromosomes are quite small. The spindle apparatus is not very distinct at metaphase. At telophase, the chromosomes clump together at the poles of the cell. Simultaneously with nuclear division, the pyrenoid divides into two daughter pyrenoids, and the daughter pyrenoids migrate towards opposite poles of the cell (figs.13, 14).

The second type of cell division, meiosis, follows the usual sequence associated with reduction division. During the early meiotic prophase, the diploid cell becomes larger, increasing in size from 5.6 microns to 7.3 microns in diameter. The chromatic material is concentrated at one side of the nucleus, and the nucleolus is located in the center of the nucleus. A definite nuclear membrane persists throughout the meiotic prophase stage. There are many granules in the cell at early prophase of first division which makes the counting of the chromosomes quite difficult. At the late meiotic prophase, the chromosomes become visible. Eight pairs of chromosomes were counted surrounding the nucleolus (fig. 15). The chromosomes clump together at metaphase so that individual chromosomes are indistinguishable. The cell appears elongated in anaphase I (fig. 16). Eight chromosomes were counted migrating towards each pole of a cell. At telophase of first division the daughter chromosomes are found grouped together at the opposite poles. Second division of meiosis follows in a regular manner, so that four haploid cells are produced from one diploid cell. The division of the pyrenoid into two daughter pyrenoids occurs simultaneously with nuclear division. The gametophytic tissue is formed as the result of mitotic divisions of the new haploid cells. This gametophytic tissue is attached to, and becomes a part of the thallus. The thallus now assumes a different morphology. The thickness of the gametophytic tissue increases from one to four or six cells. The cells form two distinct groups, larger dark green cells, and smaller pale green

ones, presenting patches of irregular shapes, all set in mosaic pattern. An outer coat covers the thallus(figs. 7, 10), and also forms septa among the groups of cells in the gametophytic region.

The haploid cells of the gametophytic tissue are smaller than the diploid somatic cells. They measure, on the average, 6 - 7 microns in diameter. Haploid cells are easily recognised by the comparatively small quantity of chromatin they contain. The smaller haploid cells contain a small chromatophore and appear light green. The larger ones contain a larger chromatophore, and are dark green in colour. Each contains a nucleus, chromatophore and pyrenoid, occupying the same relative positions as in the diploid cells. The shape of the chromatophore is axile, as in the somatic cells. The dark green haploid cells differentiate into the macro- or female gametes, and the light green ones, into the micro or male gametes. When the septa which separate groups of cells dissolve, bladder-like gametangia are formed from the outer coat of the thallus. The gametangium is ruptured, due perhaps to the mechanical pressure exerted on it by the gametes, and the gametes are released in hundreds. The macro-gamete is spherical in shape, and has no flagella. It varies in diameter from 2.4 - 4.0 microns. The micro-gamete with two flagella at its anterior end is 1.5 - 2.0 microns in diameter (figs.17,18). Each female or male gamete possesses one inconspicuous chromatophore.

Two or more micro-gametesmay approach one macro-gamete; but in this study, only one micro-gamete was observed uniting with the macro-gamete

to form a zygote (fig. 20). Motility of the zygote was observed until the gametic union was completed. Syngamy was best demonstrated in the motion picture taken during this study. The immobile zygote becomes round and enlarges to 14 or 16 microns in diameter (fig. 20). It divides mitotically into two, and then four or more cells forming a filament (figs. 20, 21), the basal cells developing into the rhizoid (figs. 22, 23). Further division of the cells in two planes results first in the formation of the young obovate thallus (figs. 22 - 25), and then of the adult thallus.

Occasionally, groups of aplanospores are found in the center and at the edge (fig. 28) of the somatic tissue. Those in this study were found in old thalli freshly collected from the sea, or growing in the culture medium left unchanged for over a month in a freezing compartment (0^0C). Groups of aplanospores were found also in thalli cultured in Lewin's medium brought to the pH of 8.8 - 9.0 (figs. 26 - 28). An aplanospore released in Provasoli's medium grew and divided in a manner similar to that of a zygote, forming first a filament and later an obovate thallus.

DISCUSSION.

Life Cycle

The life cycle observed in Prasiola meridionalis is unique in the plant kingdom. Although the alternation of generations in this species is dimorphic, the sporophytic tissue is attached to and similar to the gametophytic tissue except in two features. The gametophytic tissue is poly-stromatic whereas the sporophytic tissue is mono-stromatic. The gametophytic tissue contains green and light green reproductive patches which are not present in the sporophytic tissue.

The formation of patches containing sex cells in P. meridionalis has been reported for the first time in the present investigation. Each of the patches can be considered a gametangium. The reason for assuming so is that the outer coat which covers the whole thallus forms septa among the groups of cells. When the septa dissolve, the portion of the outer coat covering the patches becomes a bladder-like structure or a receptacle containing gametes. According to Clapper (1960) a gametangium is an enclosed compartment in which gametes are formed. Ordinarily, the term is used only when cells producing gametes are morphologically different from vegetative cells. The cells which produce gametes of P. meridionalis are haploid and they are smaller than the diploid vegetative cells. Hence the term "gametangia" may be correctly applied to the patches contained in the gametophytic tissue of P. meridionalis.

The occurrence of such patches is not limited to P. meridionalis. The presence of dark and light reproductive areas in P. japonica was reported by Yabe (1932) and Fujiyama (1955), and in P. stipitata by Drew (1957) and Friedmann (1957, 1959). Yabe stated that the male or micro-gametangium in P. japonica produces 64 gametes, and the female or macro-gametangium, 16 gametes. The patches assume various shapes in the three species, but in each case present a mosaic pattern. In P. japonica, the patches form irregular rectangles. In P. stipitata, they form smaller rectangles, and Friedmann considers them an irregular system of paired male and female areas. In P. meridionalis, the patches are irregular in shape.

There are some remarkable differences in the life cycles of the freshwater species P. japonica, and of the marine species P. meridionalis and P. stipitata. Following a detailed cytological investigation, Fujiyama (1955) concluded that P. japonica is a haplont. The term 'haplont' expresses the relative length of the haploid and diploid stages. According to Fujiyama, meiosis takes place immediately after the zygote is formed, and the plant arising from the reduction division of the zygote has the haploid number of chromosomes. The zygote, therefore, is the only diploid cell in the life cycle of the species. The formation of gametophytic or reproductive tissue in this haplont is not explained by either Yabe or Fujiyama. The life cycles of the two marine species, P. meridionalis and P. stipitata are similar to each other.

Friedmann (1959) described the life cycle of P. stipitata as being dimorphic diplo-haploidic, similar to that reported in this study for P. meridionalis.

The sporophytic generation of P. meridionalis serves two functional purposes. It provides means for both asexual and sexual reproduction by producing aplanospores and haploid cells which produce gametes after further division.

It is interesting to note that in this study the thallus produced by the germination of the diploid aplanospore, and that produced by the germination of the diploid zygote appear morphologically identical. One might expect some differences in the thalli produced from the zygote since segregation and recombination of chromosomes are associated with gamete formation and syngamy. The fact that no variations occur in the thalli may be due to homozygosity of genes responsible for these specific characters or due to rather influential environmental conditions.

There is one important characteristic common to the life cycle of the three Prasiola species : their oogamous method of sexual reproduction. A controversy existed for a long time concerning the existence of sexual reproduction in Prasiola, and various disagreements among the early wokers about its method of sexual reproduction. Both Uda (1948) and Fujiyama (1955) concluded that oogamy is the method of sexual reproduction in P. japonica. In 1957, Drew and Friedmann published a detailed account of sexual reproduction in P. stipitata.

They reported then that both male and female gametes of P. stipitata are bi-flagellate, and that the type of sexual reproduction is anisogamous. This account was followed by the conclusion of Friedmann in 1959 that both gametes are bi-flagellate. However, in 1960, Friedmann corrected the former statement when Manton, with the use of electron microscope, noted that only the male gametes possess two flagella. In all three Prasiola species only the micro-gametes are bi-flagellate.

Cytology

In the three Prasiola species studied, the diploid cells are larger than the haploid. Both haploid and diploid cells in P. stipitata are smaller than their counterparts in P. meridionalis. In P. japonica, the zygote, which is the only diploid cell, varies in size from 6 microns in diameter to 12.4 microns x 19.6 microns. The haploid cells of this species average 7 microns in diameter. The diploid cell of P. meridionalis measures 8.3 microns in diameter and the haploid, 6.7 microns.

The mitotic and meiotic cell division processes observed in Prasiola meridionalis are quite regular and similar to those reported by Friedmann (1959) in P. stipitata. The chromosome number of the three Prasiola species which have been studied cytologically are presented in Table I. It is interesting to note that the chromosome numbers differ and that P. meridionalis has the highest number of the three.

Factors Affecting Gamete Production.

Temperature, light, nutritive and climatic conditions appear to be important factors involved in the production of gametes in Prasiola.

Yabe (1932) found that sexual reproduction in P. japonica occurs generally in the months of November through April when cooler water temperature is conducive to gamete formation. Uda (1948) also found that gametes of P. japonica are released during February when the water temperature is cooler (10^0- 12^0C). Smith indicated, therefore, that there seems to be a correlation between temperature and periods in which the gametes are formed, noting that when the temperature is constantly the same throughout the year, gamete formation can take place any time. Fritsch (1955) surmised that the production of sexual cells ensues following the accumulation of nutritive material and when the climax of vegetative activity is passed. It was established in the present study that alternate periods of light and darkness induce the liberation of the gametes of P. meridionalis. Friedmann (1960) reported that mature sexual thalli of P. stipitata liberate their gametes only after they have been illuminated by fluorescent tubes.

According to Fritsch (1956) an akinete is a specialised type of vegetative reproductive structure whose cell wall is thickened 'to tide over a period unfavourable for vegetative development'. In his opinion, asexual reproduction must involve rejuvenation and division of the cell protoplast. The term "akinete" could be used in this study referring essentially to a diploid somatic cell whose protoplasm has rejuvenated and divides mitotically into 2, 4, or 8 daughter cells. The coat surrounding the daughter cells is not thickened as Fritsch has suggested. The akinete does not necessarily serve as a resting stage for P. meridionalis since the coat breaks quite easily, and the daughter cells germinate into

adult thalli without any delay. Possibly this is an asexual method of reproduction, since the protoplasm of the akinete is rejuvenated and divides into several daughter cells. The akinetes found in P. furfuracea have a special dehiscent membrane which has not been noted in other Prasiola species. The akinetes of P. stipitata described by Friedmann (1959) are similar to those found in P. meridionalis.

SUMMARY.

1. Provasoli's medium (containing kinetin, adenine, indole acetic acid, gibberelin and vitamins) brought to a pH of 8.2 - 8.4, at temperatures of 3^0 - 5^0C, supports good growth of all stages of development of Prasiola meridionalis in culture.
2. P. meridionalis is a diplo-haploid, dimorphic plant.
3. Mature diploid cells at the apex of the thallus divide by meiosis, each producing four haploid cells.
4. After mitotic divisions, the haploid cells form the gametophytic tissue which becomes a continuation of the somatic tissue within the same thallus.
5. The gametophytic tissue is poly-stromatic and the rest of the thallus (the rhizoid and somatic tissues) is mono-stromatic.
6. Patches of deep green cells, containing potential macro-gametes, alternate with patches of cells of very light colour which produce micro-gametes. Hence a mosaic pattern is formed at the apex of the thallus.
7. Sexual reproduction is oogamous. The female is spherical and has no flagella; but the male gamete, which is smaller in size, has two flagella at the anterior end.
8. Sixteen chromosomes were counted in the diploid cells of P. meridionalis and eight in haploid cells.
9. Prasiola meridionalis reproduces asexually through germination of aplanospores.

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TABLE I. - CHROMOSOME NUMBERS IN PRASIOLA

	diploid	haploid	reference	
P. japonica	-	3	Fujiyama	1955
P. stipitata	12	6	Friedmann	1959
P. meridionalis	16	8	Akintobi	1961

ABBREVIATIONS.

ak	.....	akinete (asexual cells)
ap	.....	aplanospore
b	.....	blade
c	.....	chromosome(s)
ch	.....	chromatophore
cp	.....	cell plate
cw	.....	inner coat or individual cell wall
f	.....	flagella
gt	.....	gametophytic tissue
mag	.....	macro-gamete
mig	.....	micro-gamete
n	.....	nucleus
nl	.....	nucleolus
oc	.....	outer coat
p	.....	pyrenoid
r	.....	rhizoid
s	.....	stipe
st	.....	somatic tissue
z	.....	zygote

Figure 1. Culture of *Prasiola meridionalis* in petri dish. $x^2/3$.

Figure 2. An adult *P. meridionalis* thallus.
Note rhizoid, somatic tissue and gametophytic tissue.
x20.

FIGS. 1 & 2 - PRASIOLA MERIDIONALIS THALLI

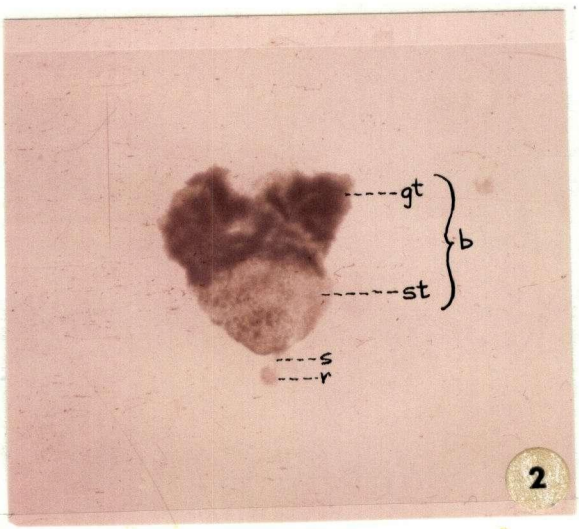


FIG.3 - ALTERNATION OF GENERATIONS IN PRASIOLA MERIDIONALIS.

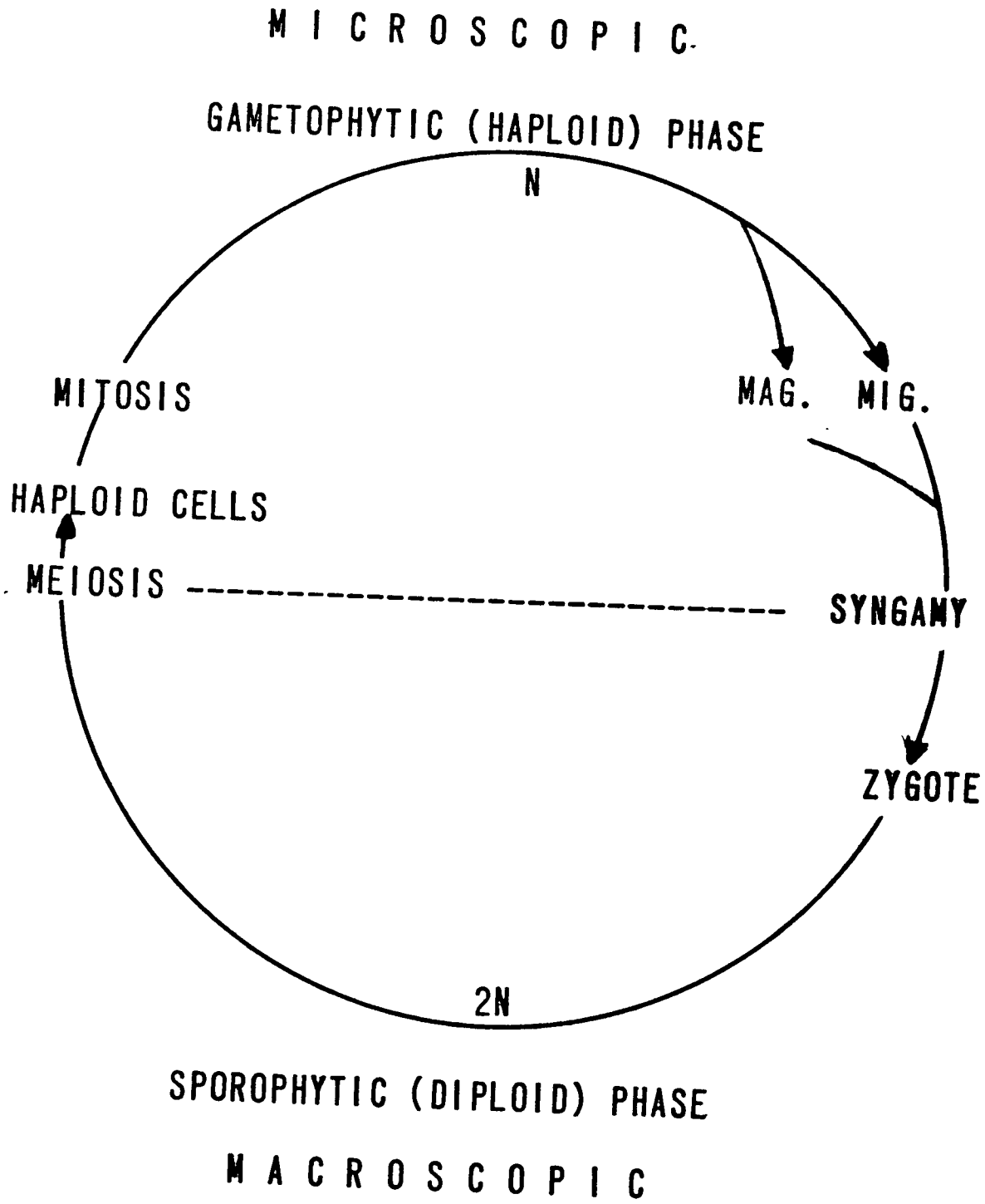
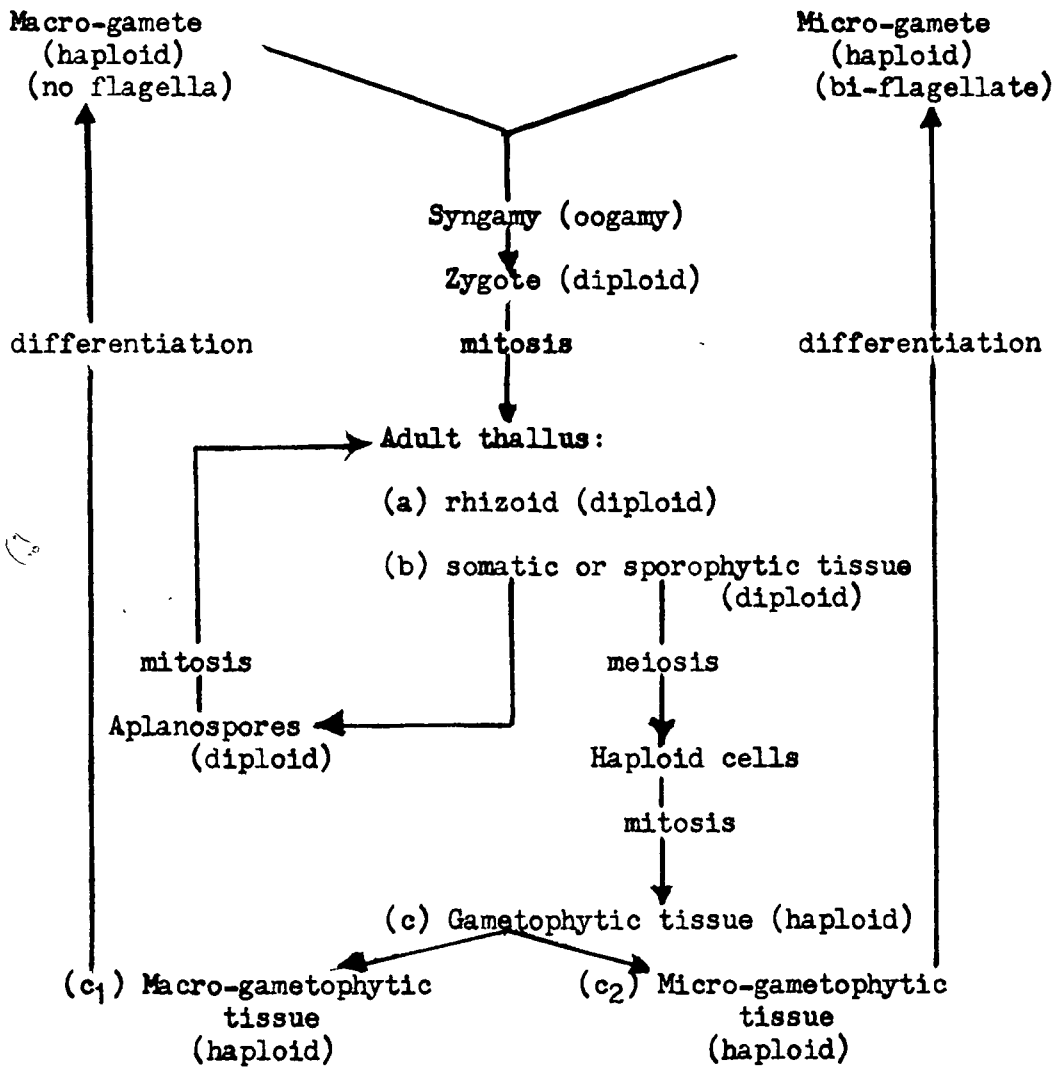


FIG.4. LIFE CYCLE OF PRASIOLA MERIDIONALIS.



Figures 5 - 10. Morphology of *Prasiola meridionalis*

Fig. 5 Surface view of the thallus of *P. meridionalis*. Note the rhizoid and blade. x5

Fig. 6 Whole thallus of *P. meridionalis*. Note the forked rhizoid. The gametophytic tissue with very light and dark areas is evident at the apex. x25

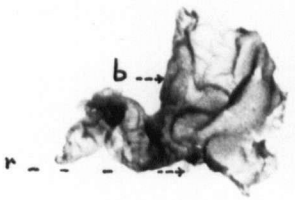
Fig. 7 Surface view of the flat rhizoid, short stipe, somatic tissue, and the mucilaginous material secreted by the basal cells. A common coat covers the thallus. The cells of the young thallus are shaped and arranged irregularly. x1800

Fig. 8 The cells of the somatic tissue. Note the asexual cells within the center of the thallus. x1500

Fig. 9 Mature cells of somatic tissue. Dark spots in the cells are lobes of chromatophores. x850

Fig. 10 Camera lucida drawing of a young thallus showing patches formed by the gametophytic tissue. The dark patches contain cells which differentiate into macro-gametes, and the light ones, micro-gametes. x25

FIGS. 5 - 10. MORPHOLOGY OF PRASIOLA MERIDIONALIS



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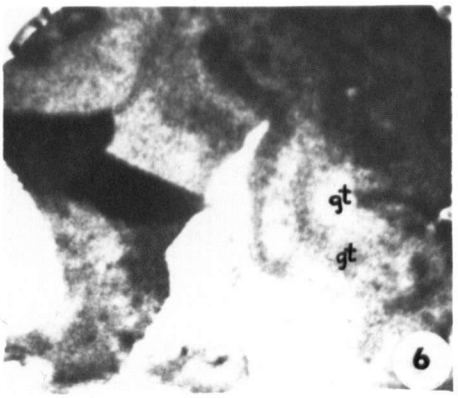

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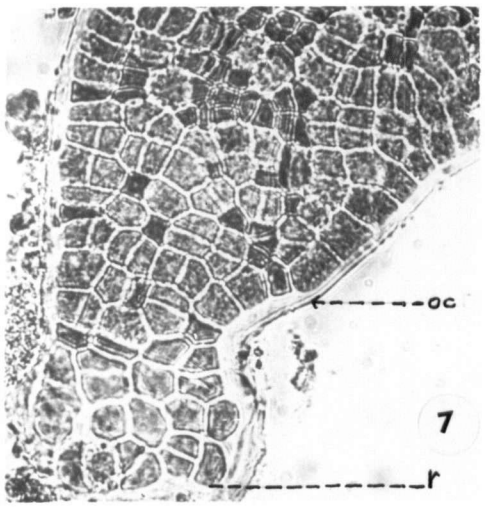

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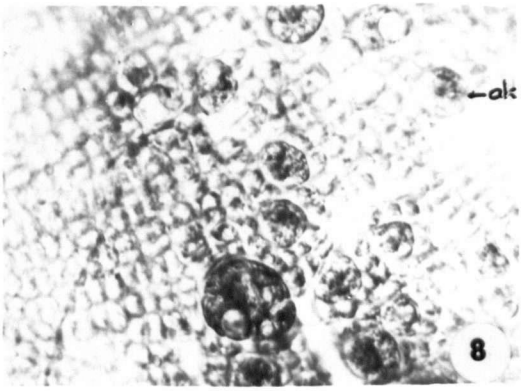

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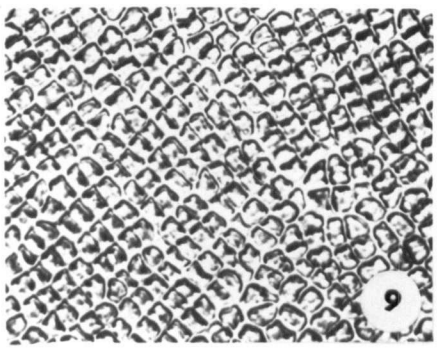

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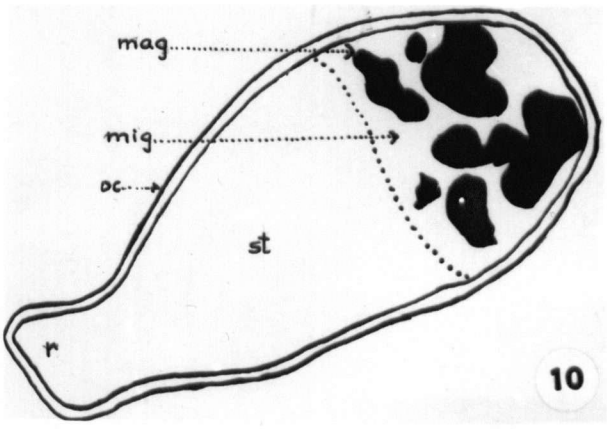

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Figures 11 - 16. Cytology of Prasiola meridionalis

Fig. 11 Typical cells of sporophytic tissue of P. meridionalis. Note the relative positions of the nucleus and pyrenoid. x1800

Fig. 12 Drawing of mitotic anaphase. Sixteen chromosomes are present at each pole. x1800

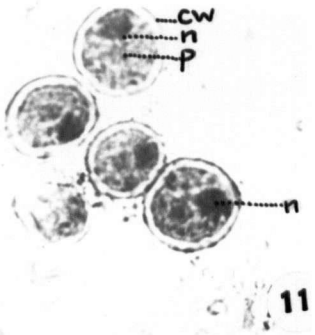
Fig. 13 Division of both nucleus and pyrenoid. x1800

Fig. 14 Camera lucida drawing of fig. 13. x2300
Sixteen chromosomes are evident at each pole of the cell with the cell plate in between.

Fig. 15 Camera lucida drawing of mitotic prophase in cells from the gametophytic tissue. Eight chromosomes are located close to the periphery of the nuclear membrane. x1800

Fig. 16 Camera lucida drawing of mitotic anaphase in a cell of the gametophytic tissue. Note eight chromosomes moving towards each pole of the elongated cell. x1800

FIGS. 11 - 16. CYTOLOGY OF PRASIOLA MERIDIONALIS



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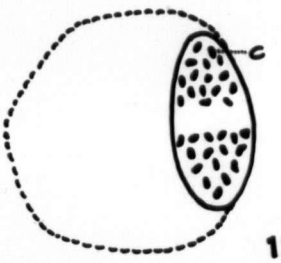

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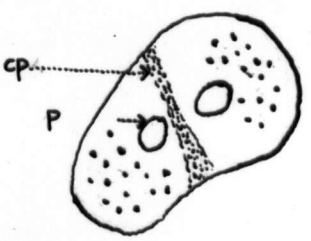

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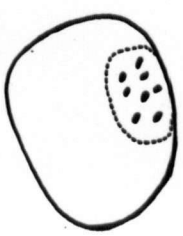

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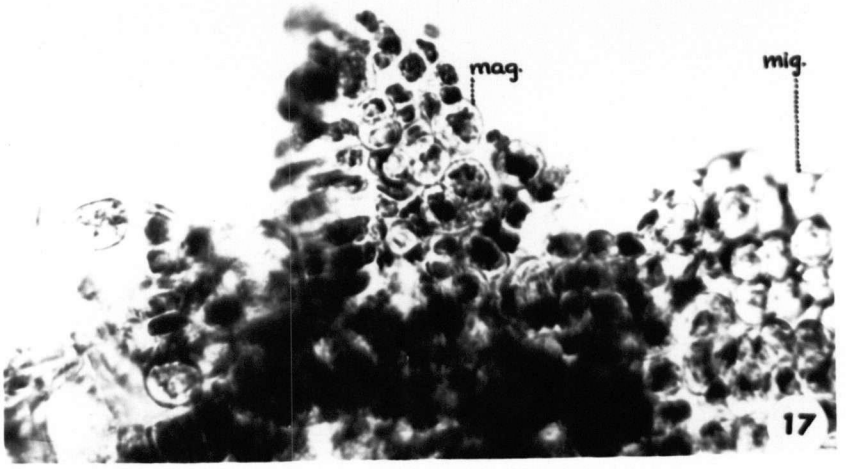
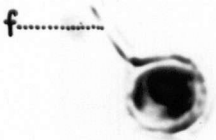
Figures 17 & 18. Gametes of Prasiola meridionalis.

Fig. 17 Gametes escaping from ruptured gametangia. Note the micro-gamete on the right and the macro-gamete at the top center of the photograph. x2800

Fig. 18 Male gamete with two flagella. x4300

FIGS. 17 & 18. GAMETES OF PRASIOLA MERIDIONALIS





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Figures 19 - 23. Stages in the life cycle of Prasiola meridionalis

Fig. 19 Fusion of larger macro-gamete and smaller micro-gametes. Printed from a 16 mm. cine frame. x1800

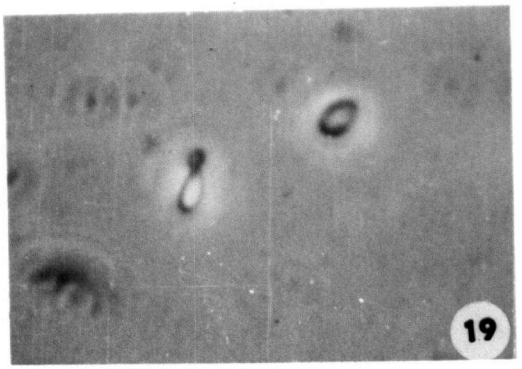
Fig. 20 Incomplete mitotic division of the zygote showing separate chromatophores. x1800

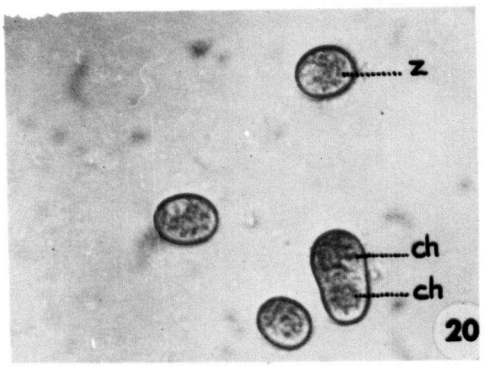
Fig. 21 A uniseriate filament resulting from mitotic divisions of the zygote in one plane. Cell plates are visible. 1800

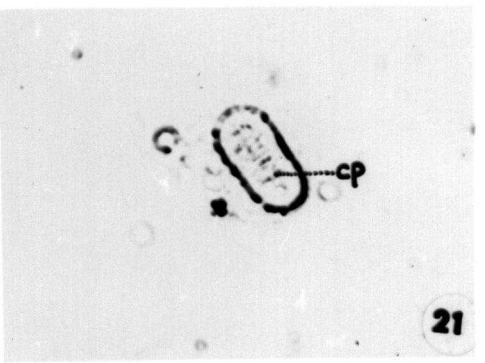
Fig. 22 Young thallus. Note that basal cells have formed a rhizoid and the common outer coat covers the whole thallus. x1800

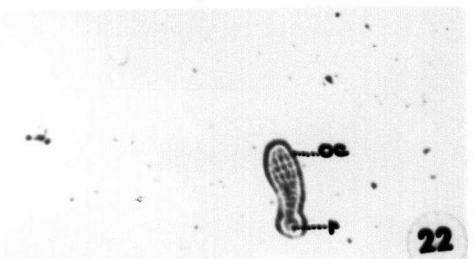
Fig. 23 Thallus pictured in fig. 22, 16 days older. The rhizoid has become forked. The outer coat is present but shows faintly. x1800

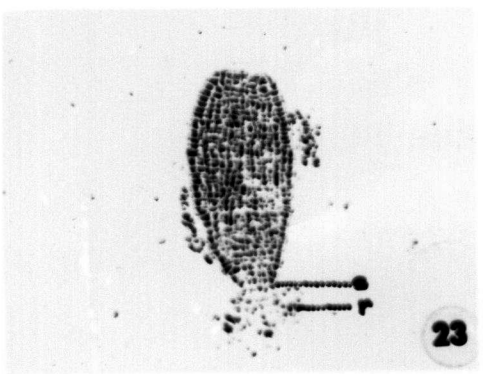
FIGS. 19 - 23. STAGES IN THE LIFE CYCLE OF PRASIOLA MERIDIONALIS.











Figures 24 - 28. Stages of growth of Prasiola meridionalis and asexual reproduction.

Fig. 24 Young thallus - 12 weeks old. Note the common coat. x500

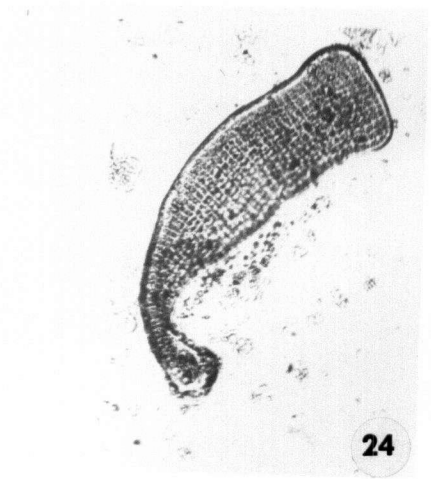
Fig. 25 Adult thallus with mucilaginous material at the base of the rhizoid. x500

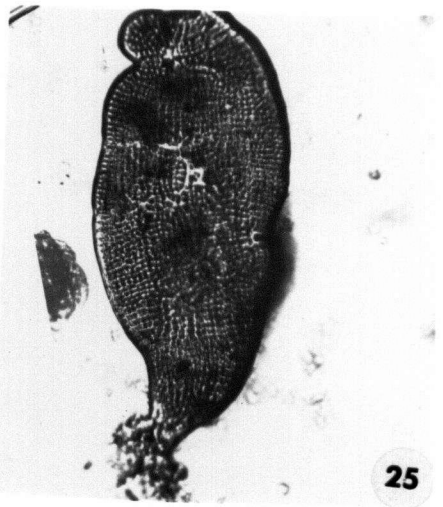
Fig. 26 Mitotic division of the mature diploid cell, forming aplanospores. At this stage the four chromatophores are incompletely partitioned by the cell plates. x2800

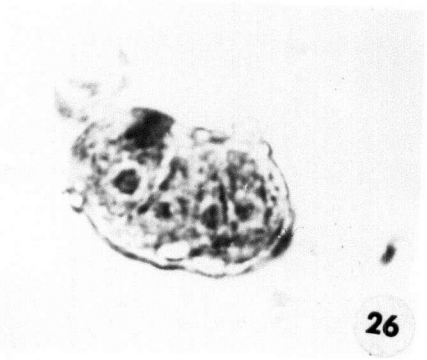
Fig. 27 An advanced stage of fig. 26. Each aplanospore (within the mother cell coat)has a pyrenoid, nucleus and a thin cell wall. x2100

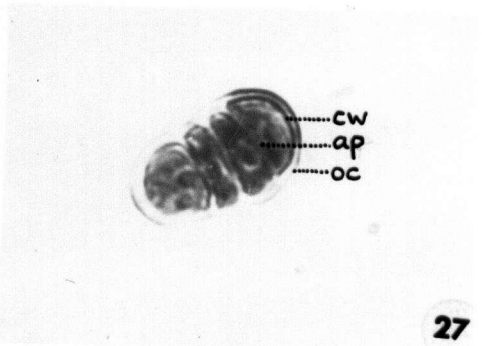
Fig. 28 Four, six, or eight aplanospores within a common coat. x1500

FIGS. 24 - 28. STAGES OF GROWTH OF PRASIOLA MERIDIONALIS AND ASEXUAL REPRODUCTION.









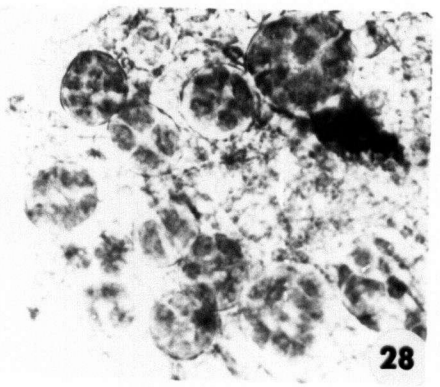


FIG.3 - ALTERNATION OF GENERATIONS IN PRASIOLA MERIDIONALIS.

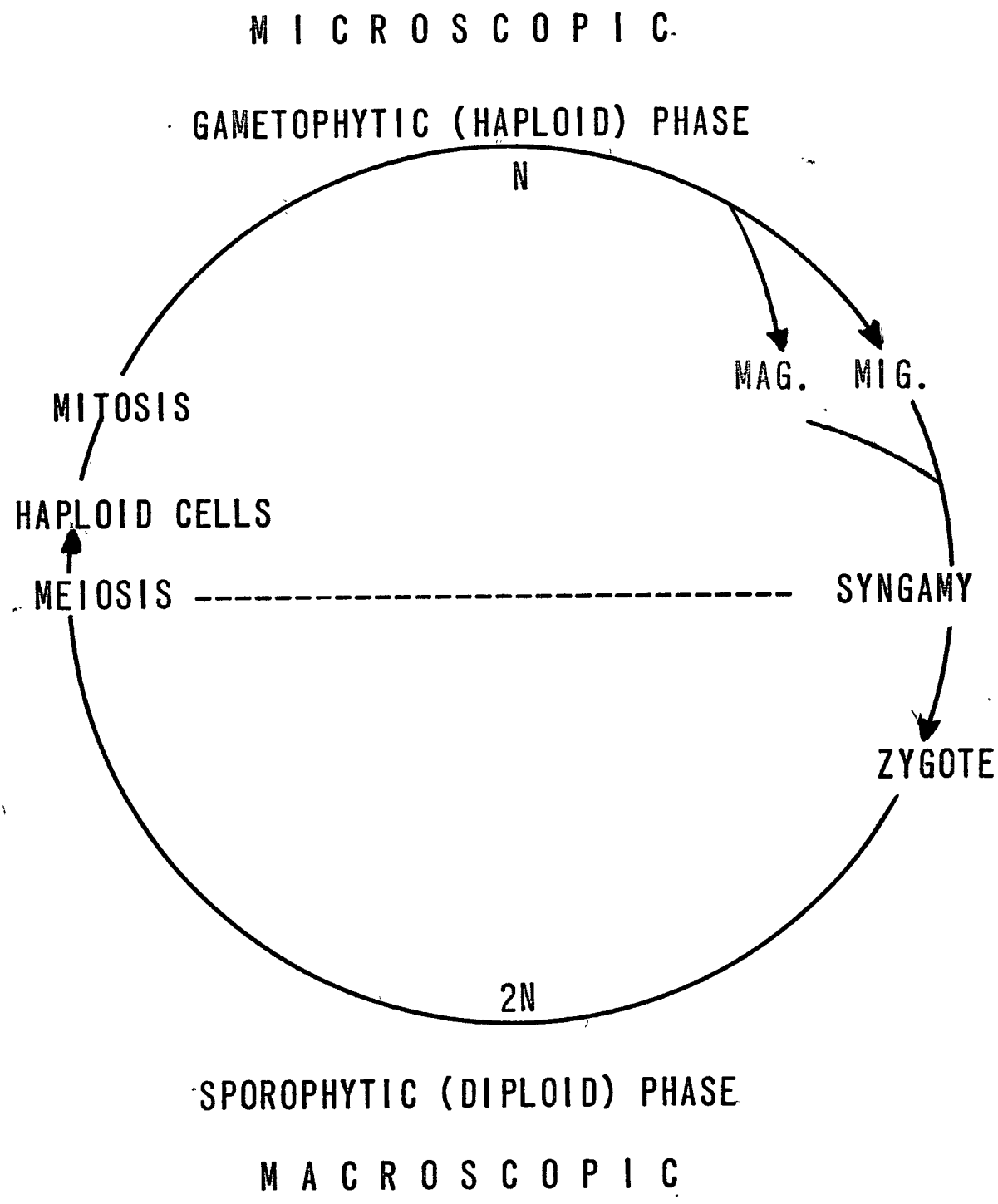


FIG.4. LIFE CYCLE OF PRASIOLA MERIDIONALIS.