The Life-History and Reproduction of *Polyides caprinus* (Gunn.) Papenf.

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With Plate VI and six Figures in the Text

Abstract

This paper deals with the development of the sexual organs (in their respective nemathecia), the carposporophyte and the tetrasporophyte of *Polyides caprinus* in the British Isles. The carposporophyte is of the 'diffuse type' and resembles that of some other Cryptonemiales and some Gigartinales (*sensu* Kylin) in essential features. The life-history is of the *Polysiphonia*-type on the coast of Wales, as male, female, and tetrasporic plants occur in nature and reduction division takes place in the tetrasporangium.

Table of Contents

		PAGE
1.	Introduction	211
2.	Material and Methods	212
3.	Development of Sexual Reproductive Organs of the Gametophyte	213
4.	Development of the Carposporophyte	217
5.	Reduction Division in the Tetrasporangium and other Cytological Observations	222
6.	Early Stages in the Germination of Spores	224
7.	Discussion	225
8.	Summary	228
	Literature Cited	229

1. Introduction

POLYIDES CAPRINUS is the only species of this genus which was included by Schmitz and Hauptfleisch (1897) in the family Rhizophyllidaceae. Later Kylin (1944) moved the genus to the new family the Polyideaceae without giving any diagnostic family characters, leaving this new family, as well as the older one, in the Cryptonemiales. The family contains the two genera *Polyides* and *Rhodopeltis*, and the distinguishing characters appear to be based on those of the former and the better-known genus.

P. caprinus is confined to the colder latitudes of the northern hemisphere and occurs commonly in the inter-tidal rock-pools of many shores of the British Isles. It is a dark-purple or purple-red lithophyte of cartilaginous texture when fresh, but becomes horny and black when dry. The repeatedly

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dichotomously branched upright cylindrical fronds arise from a perennial disk of varying size. Growth of the thallus is brought about by a number of apical cells and the mature thallus is differentiated into a 'medulla', an inner and an outer 'cortex'.

In the British Isles, male, female, and tetrasporic thalli occur and so there is no reason to doubt that the life-history is of the *Polysiphonia*-type. On the other hand, the preponderance of sexual plants of *P. caprinus* in Denmark (Rosenvinge, 1917), the absence of tetrasporic plants on the west coast of Sweden (Kylin, 1923), and the occurrence of tetrasporangia on sexual individuals (Sinova, 1912) are suggestive of a deviation from the *Polysiphonia*-type of life-history.

Although there have been several investigations of carposporophytes of species belonging to the Cryptonemiales, comparatively few are as detailed as is desirable. Least is known of the nemathecial types. Our existing knowledge of the development of the sexual organs and carposporophyte of *P. caprinus* dates back to the classical researches of Thuret and Bornet (1878), subsequent investigators including Rosenvinge (1917) and Kylin (1923) having contributed little. The carposporophyte appears, on the basis of the available information, to be of the type general in the Cryptonemiales. Excepting the Corallinaceae, the carposporophyte of the Cryptonemiales conforms in general to a uniform pattern, being of the 'diffuse type'. One or more gonimoblasts develop either directly from the fertilized carpogonium or from certain cells of the carpogonial branch with which the fertilized carpogonium has previously communicated. The auxiliary cell branches are well separated from the fertile branches.

The only cytological investigations of any extent in this large Order are those of Yamanouchi (1921) on *Corallina officinalis* var. *mediterranea* and Suneson (1950) on species of *Lithophyllum*.

For these reasons an investigation of the life-history and reproduction of *Polyides caprinus* seemed desirable.

2. Material and Methods

Most of the material of *P. caprinus* used in this investigation has been collected at frequent intervals over a period of one year from Rhosneigr on the west coast of Anglesey Island (North Wales), but in addition valuable collections have been received from other parts of the United Kingdom.

Plants required for cytological observations have been fixed on the shore, only such plants as were completely submerged under water being used. Further material has also been brought to the laboratory and fixed at different times of the day. The maximum number of nuclei in division has been found in plants fixed on the shore between 12.30 p.m. and 2.30 p.m. when low water was approximately 3–4 hours later.

Material has been fixed in a variety of fixatives, but the best results have been given by formalin-alcohol (Drew, 1934) and formalin-acetic-alcohol

(Westbrook, 1928). Whatever be the type of fixative employed, the material has had to be softened before being squashed or sectioned. The method for softening described by Drew (1945) has been followed. Kylin's (1923) method for softening material has also been used for both fresh and fixed material to be stained subsequently with Gram's iodine solution.

Free-hand sections and serial microtome sections (6–24 μ thick) have been employed in the study of nemathecia and carposporophytes. Squash preparations (E. Greig-Smith, unpub.) have been found, however, to be very much more useful in studying meiosis in the tetrasporangium and the organization of the carposporophyte.

Out of a number of cytoplasmic and nuclear stains tried, Brazilin (Drew, unpub.), and Feulgen's reagent proved the best for cytological purposes and Gram's iodine for dissections of nemathecia used for studying the development and organization of the carposporophyte.

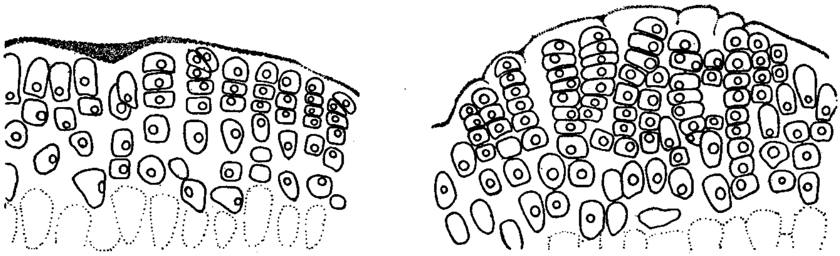
3. Development of the Sexual Reproductive Organs of the Gametophyte

Early stages in the development of male and female nemathecia, which then look alike, have been observed in plants collected during August and September. When first recognizable the nemathecia appear to the naked eye as small, pale yellow, pink or purple, oval or circular patches of different sizes. Transverse and longitudinal sections of such pieces of thalli reveal that nemathecia are formed from groups of apical cells of the cortex. (Text-fig. 1.) The number of cells concerned in the formation of each group and the position of the nemathecia on the thallus are both highly variable. Nothing is known about the factors responsible for the stimulation of the apical cells into active division, nor is any information available at present regarding the factors that control such a fortuitous development of these fertile tracts on the thallus. The cells which give rise to nemathecia are first recognizable by their greater size, large nuclei, and homogeneous protoplasm. The nucleus is near the basal wall and the cells divide in quick succession giving rise to short, compactly arranged parallel filaments composed of discoid cells which are broader than long (Text-fig. 1, B).

The development of the male and female nemathecial filaments differs slightly from this stage onwards. The distal cells of the former undergo periclinal divisions resulting in the divergence of the filaments. Large, fully developed male nemathecia may cover the entire surface of the upper dichotomies of the thalli.

While the male nemathecial filaments pierce through the cuticle and diverge at a very early stage, those of the female nemathecium grow and branch parallel for a while, lifting the cuticle ahead of them by their concerted growth. However, patches of the original cuticle of the thallus with numerous epiphytic diatoms attached may sometimes be seen included between the bases of the fully grown nemathecial filaments.

The cells of the filaments very soon become quadrate in section and contain a proportionately large nucleus in the lightly staining cytoplasm. When the sterile nemathecial filaments reach a length of about 10–12 cells, short side branches begin to develop, usually 3 or 4 cells below the apical cell. All the cells of the nemathecial filaments by now contain many small floridean starch grains. The increase in length of the nemathecial filaments is due to the activity of the apical cells and the elongation of the cells thus formed. Each nemathecial filament has its own transparent mucilaginous sheath, but at a later stage those parts covering the filament apices coalesce to give a continuous canopy-like covering which appears to toughen and thicken with age. In old nemathecia the lower surface of this covering shows a honeycomb-like



TEXT-FIG. 1. A. Part of a transverse section of a female plant showing the formation of nemathecial filaments. Some of the apical cells to the left are about to divide. B. A slightly later stage than A. The mucilaginous sheaths of individual filaments are visible at the apices. (Brazilin.) ×566.

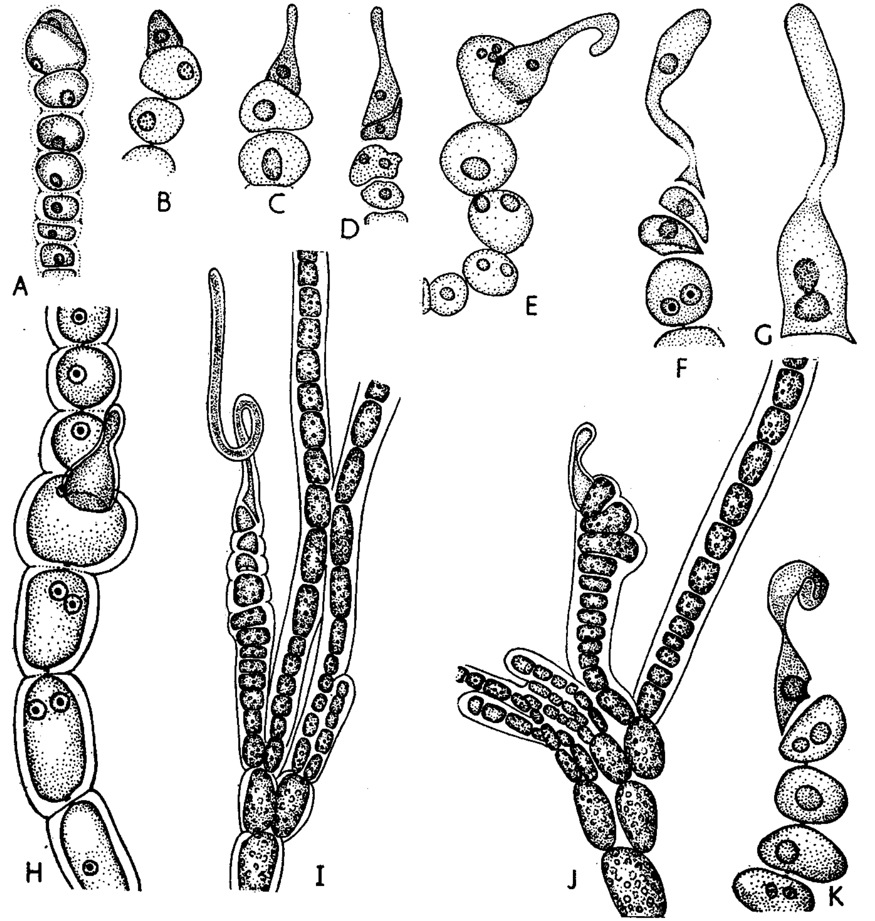
pattern (seen frequently in squashes) with the apices of filaments fitting into the circular or polygonal areas. Lateral extension of nemathecia takes place by the continued production of more nemathecial filaments at the margin of those already initiated. The centrifugal spreading of adjacent nemathecia may lead to their fusion and eventually to the formation of large nemathecia extending completely around the thallus. Ultimately when the carpospores have been shed nemathecial filaments are cast off.

All the filaments of male nemathecia are fertile and they grow to varying lengths before the cells undergo longitudinal divisions. Such divisions never occur in the basal 3 or 4 cells which elongate considerably. The proximal axial cells give rise to one or two mother-cells, while the distal ones form 3 or 4. Usually three, but sometimes one, two, or four spermatangia are borne by each mother-cell. The contents of the spermatangium are liberated as a single spherical colourless spermatium. The nucleus of the liberated spermatium is in prophase, and usually surrounded by a narrow zone of cytoplasm. The delicate, transparent wall of the liberated spermatium is clearly seen in preparations stained with cytoplasmic stains and in spermatia which are attached to trichogynes. The account of the development of spermatia is in close agreement with the accounts of Schmitz (1883) and Kylin (1923). Neither of these authors, however, describes the development of such long brush-like branchlets as have been found during this investigation.

Only certain filaments of the female nemathecia bear carpogonia. When the sterile nemathecial filaments are ten to twelve cells long and the cells of some filaments begin to enlarge, certain other slightly shorter filaments become arrested in their growth. These shorter filaments function as fertile branches and bear carpogonia. Since they are slightly curved and the trichogynes are usually twisted, the entire branch is seldom obtained in a single section. In following the development of the fertile branch, therefore, use has been made not only of serial microtome sections but also of squash preparations as well as dissections stained with iodine.

The number of cells in a fertile branch is very variable, the earliest formed branches containing fewer cells than the later formed branches. As the production of carpogonia continues throughout the existence of nemathecia, all stages in their development can be seen even in nemathecia containing mature carposporophytes. The apical cell of the fertile branch divides by an oblique wall into a small upper cell, the carpogonium-initial, and a large lower one, the hypogynous cell (Text-fig. 2, A). Subsequently the apical region of the apical cell elongates into a trichogyne which at no stage in its development has been observed to possess a nucleus. In a few cases the trichogyne begins to develop before the carpogonium is separated from the hypogynous cell (Text-fig. 2, D). The carpogonia are therefore apical and sessile, but in some instances the wall between the carpogonium and hypogynous cell is so oblique that the carpogonium appears to be lateral. The mucilaginous sheath surrounding the carpogonium and trichogyne is a continuation of the sheath of the filament.

Carpogonia with forked trichogynes or two separate trichogynes have sometimes been observed. In a single example two carpogonia had arisen from the same hypogynous cell and hence one must have been lateral. Sometimes the development of the carpogonium appears to be arrested very early and it may then be transformed into a long or short hyaline hair. Since the carpogonia which are formed early in the development of the nemathecium terminate filaments almost as long as the sterile nemathecial filaments, a comparatively short trichogyne reaches to the outer surface. The later-formed carpogonia, however, are situated at varying distances from the surface of the nemathecium and the trichogynes of those which terminate short branches only reach the surface if very long. It appears likely, therefore, that only the earliest-formed carpogonia of the nemathecia with short trichogynes are fertilized. Moreover, their maturation synchronizes with the development of spermatangia. At the time of fertilization the trichogyne either just reaches the surface or projects slightly beyond. The cytoplasm stains deeply and a single small nucleus is to be seen in the basal region of the carpogonium. In the majority of instances spermatia have been seen attached to the apical gelatinized region of the trichogyne and rarely to its sides as figured by Thuret and Bornet (1878). By the time the gonimoblast is formed, spermatia can no longer be seen still attached to the trichogyne as figured by Thuret and Bornet (1878), Pl. XXXVIII, Figs. 15, 16, and 18. This present investiga-



TEXT-FIG. 2. A. Carpogonium-initial formed by the oblique division of the apical cell of a fertile branch. All cells of the branch are uninucleate. B. Conical carpogonium-initial. C. Trichogyne slightly elongated. D. Carpogonium-initial divided to form the hypogynous cell after the trichogyne has elongated. E. Sessile lateral carpogonium; hypogynous cell four-nucleate. F. Carpogonium showing what is presumably the male nucleus in the trichogyne. G. Early stage of fusion of male and female nuclei within the carpogonium. H. Fertile branch showing an apparently lateral, sessile carpogonium due to formation of lateral branch from hypogynous cell. I and J. Fertile branches from old nemathecia; in I the trichogyne has elongated and three cells below the carpogonium are depleted of floridean starch. J. Carpogonium nearly lateral and trichogyne not yet elongated. K. Fertilization nucleus. Neck of the trichogyne greatly constricted. (I and J. Iodine; E. Feulgen; the rest Brazilin.) G ×1700; I and J ×425; the rest ×850.

tion has led the writer to question whether this may not be a composite figure therefore. The entry of the spermatium nucleus into the trichogyne has not been seen. However, in a number of examples a deeply staining nucleus has been seen at various positions within the trichogyne. It is assumed that these are male nuclei on account of the close resemblance between the nuclei of the spermatia and such nuclei seen in trichogynes. (Pl. VI, Fig. 7.) In a few examples two small nuclei, presumably the male and female gametic nuclei, have been observed in the basal region of the carpogonium (Text-fig. 2, G, Pl. VI, Fig. 8). A deeply stained large nucleus filling most of the basal region of the carpogonium has been found in still other examples and it is presumed that the large nucleus represents the fertilization nucleus (Text-fig. 2, K). The trichogynes of what appear to be both fertilized and unfertilized carpogonia persist. The trichogyne becomes broad and contracted after fertilization and sometimes bent and coiled. The neck region of the trichogyne becomes greatly constricted (Text-fig. 2, K; Pl. VI, Fig. 8).

At the time the carpogonium is initiated all the cells of the fertile branch are uninucleate and full of floridean starch grains. At a later stage, a variable number of the cells in the upper part of the branch become swollen and protruberances develop. All the protruberances may be on one side or be arranged spirally. Frequently, one or more cells of the fertile branch bear one- or two-celled branchlets. In a few examples seen the hypogynous cell had branched, the carpogonium having been pushed to one side and thus appearing lateral. Meanwhile, the number of nuclei in the cells of the fertile branches increases to two, three, or four (Text-fig. 2, E).

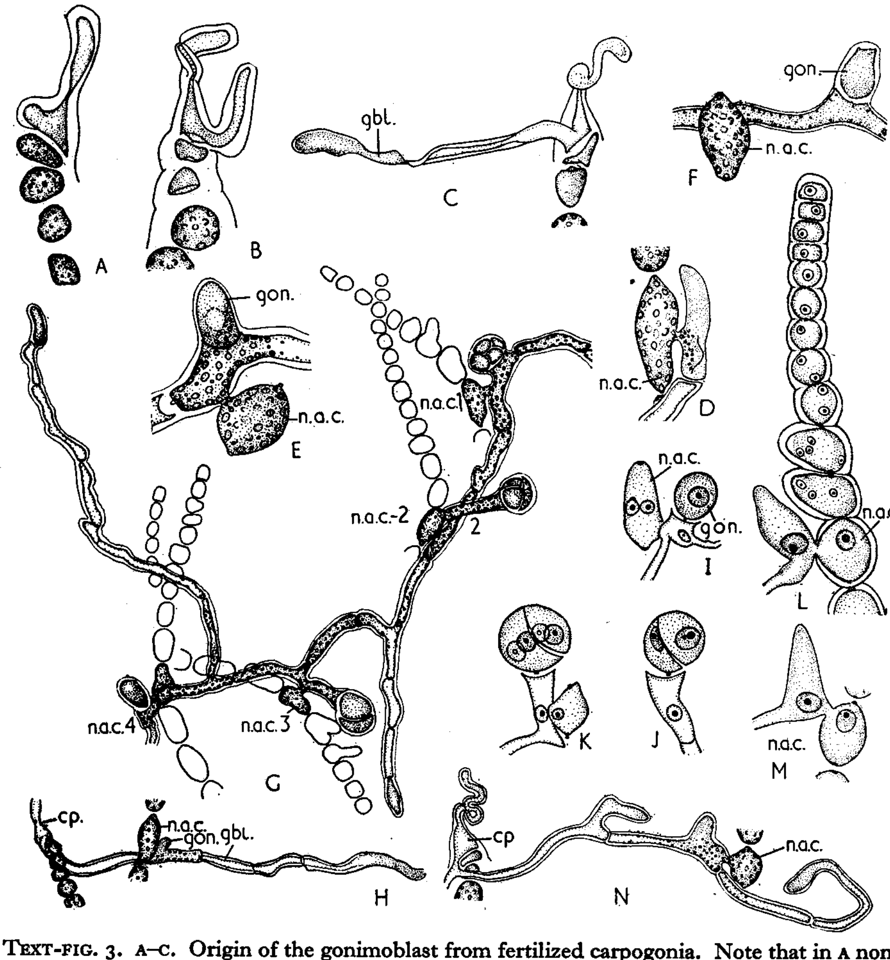
The cells of fertile branches bearing carpogonia with very elongated trichogynes are often small, shrivelled, and mis-shapen. In addition, the cytoplasm of as many as seven cells beneath the carpogonium is refringent and homogenous, floridean starch being absent. The disappearance of the starch starts in the hypogynous cell and progresses down the branch.

4. Development of the Carposporophyte[1]

The fertilized carpogonium gives rise direct and without fusion with any other cell of the fertile branch to a single broad outgrowth, the gonimoblast, which may be directed upwards, sidewards, or downwards (Text-fig. 3, B–C). The gonimoblast elongates following an undulating course between the lower part of the closely-set nemathecial filaments. In consequence, it is difficult to follow it in sections and the following account is based mainly on squash preparations. At first the whole gonimoblast contains dense cytoplasm, but as it elongates, this is confined to the apical region only. The dark-staining nucleus is situated in this cytoplasm a short distance from the apex. (Pl. VI, Fig. 12.)

As the gonimoblast grows and branches, it fuses successively with a number of certain intercalary cells of some of the nemathecial filaments. These cells are indistinguishable from other cells of the nemathecial filaments and are more or less at the same distance from the surface as are the carpogonia. In spite

[1] The terminology used in this section is that proposed by Drew (1954).



TEXT-FIG. 3. A–C. Origin of the gonimoblast from fertilized carpogonia. Note that in A none of the cells of the fertile branch are depleted of floridean starch. D. Early stage in development of gonimolobe-initial after fusion of gonimoblast with nutritive auxiliary cell. E. Gonimolobe-initial formed from the gonimoblast in a lateral position. F. Gonimolobe-initial formed slightly away from the region of contact with the nutritive auxiliary cell. G. Part of a carposporophyte showing the fusion of one gonimoblast with four nutritive auxiliary cells (1–4). Contents of the auxiliary cells only are shown, although the other cells are also full of floridean starch. H. Well-developed gonimoblast showing connexion with the carpogonium. No fusions between the carpogonium and the cells of the fertile branch. Fusion of the gonimoblast with an auxiliary cell and the early appearance of a gonimolobe-initial seen. I–K. Three stages in the division of the gonimolobe-initial. L and M. Early stages in the development of gonimolobe-initials from the point of fusion of a gonimoblast with an auxiliary cell. Auxiliary cell uninucleate. N. Young gonimoblast still showing connexion with carpogonium, and fusion with a nutritive auxiliary cell and the formation of two branches, one near the carpogonium and the other between the carpogonium and the nutritive auxiliary cell.

cp, carpogonium; *gbl*, gonimoblast; *n.a.c.*, nutritive auxiliary cell; *gon.*, gonimolobe-initial. (I–M, Brazilin; the rest iodine.) G, H, N $\times 285$; the rest $\times 566$.

of repeated efforts to find examples of the stages in fusion between a gonimoblast and such a cell, none have been seen. Being very delicate structures the earliest stages of fusion do not survive the pressure applied in squashing. Slightly more advanced stages showing open communication between the auxiliary cell and gonimoblast have sometimes been seen. In no example observed during this investigation has there been any indication of the gonimoblast nucleus entering the auxiliary cell (Pl. VI, Fig. 11). Using Drew's (1954) terminology, they are therefore nutritive auxiliary cells. After fusion with the first nutritive auxiliary cell, the gonimoblast grows on to fuse with other similar cells at varying distances from each other. As the gonimoblast lengthens it becomes considerably branched, increases in diameter, and septa can be clearly seen. Pit-connexions are visible in the transverse walls and the longitudinal walls are usually provided with short stumpy protrusions. The cells of the gonimoblast contain starchy food especially in the vicinity of the nutritive auxiliary cells. By contrast, the auxiliary cells lose their floridean starch (Text-fig. 3, G–M).

The formation of special spore-producing lateral branches of the gonimoblast is initiated by the fusion of the gonimoblast and the nutritive auxiliary cells. During its growth the gonimoblast fuses with a number of nutritive auxiliary cells, but whereas the formation of the spore-producing lateral branches is limited to the region of the fusions in the early stages of the development of the carposporophyte, this is not so in the later stages, the spore-producing laterals (the gonimolobes) being formed remote from nutritive auxiliary cells. The gonimolobes of any one carposporophyte therefore outnumber the nutritive auxiliary cells. These spore-forming lateral branches, each of which forms a gonimolobe, are formed from the gonimoblast and not the auxiliary cell in one of the following ways:

1. The tip of the main gonimoblast may be cut off as a gonimolobe-initial and a branch which continues the growth of the gonimoblast may arise just near the region of contact with the auxiliary cell (Text-fig. 3, G at 3).

2. A short branch may arise from the enlarged communication tube between the gonimoblast and the auxiliary cell and its tip is cut off as a gonimolobe-initial (Text-fig. 3, G at 2).

3. A gonimolobe-initial may terminate a short lateral branch of the main gonimoblast either near the place of fusion with the nutritive auxiliary cell or slightly farther away from it (Text-fig. 3, G at 4, and F).

The gonimolobe-initial contains homogeneous dense cytoplasm and a large nucleus (Text-fig. 3, I). The initial enlarges and divides by successive walls into a large central cell and generally three or rarely four small peripheral cells separated by curved walls. The peripheral cells act as apical cells and divide by walls at right angles to the basal walls. The cells thus formed divide repeatedly until a spherical cluster of cells is formed. Floridean starch accumulates in all these cells and eventually the peripheral cells of the developing gonimolobe filaments enlarge and form obconical carposporangia.

The latter are generally uninucleate, but sometimes they may be two- or three-nucleate. A section of a mature gonimolobe shows the central cell surrounded by a number of small cells, the placenta, and a peripheral zone of carposporangia, the whole cluster being enclosed in a firm transparent mucilaginous membrane.

If several carpogonia in a nemathecium are fertilized, a corresponding number of carposporophytes will develop, but even if several carposporophytes are present they cannot be distinguished individually.

As has been stated, the gonimoblast is formed without any prior fusion of the carpogonium with cells of the fertile branch. However, in twelve examples such fusions have been seen. They were found in nemathecia containing gonimolobes in an advanced stage of development and the evidence suggests that they should be regarded as secondary fusions occurring after the development of the gonimoblast. This interpretation is supported by the example shown in Text-fig. 4, A, in which a branch from the base of the gonimoblast has apparently grown down and fused with the third and fourth cells of the fertile branch. From the down-growing portion of the gonimoblast one branch has arisen subsequently. These secondary fusions appear to have taken place with those cells of the branch immediately beneath the depleted cells.

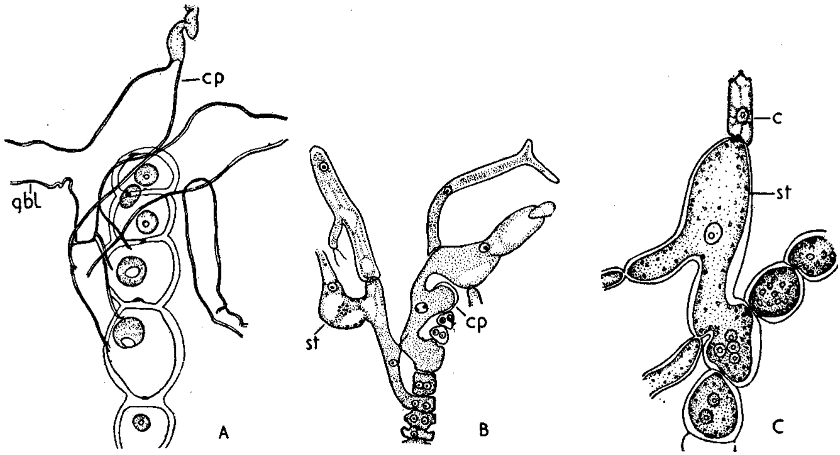
Another interesting example is shown in Text-fig. 4, B, and in this fusion has taken place with the third and fifth cells of the fertile branch. From the point of fusion with the fifth cell there appears to have arisen a branch which has given rise near by to a gonimolobe and then continued its growth. The stalk cell of the gonimolobe from which the spores have been liberated is seen as a dilated cell (*st*) in the figure. A comparison of the sizes of the fertile branches shown in Text-fig. 3, A–C and this example suggests that these are secondary fusions which take place late in the development of the gonimoblast.

Thuret and Bornet (1878) considered that such fusions between the carpogonium and the cells of the branch below take place before the formation of the gonimoblast. The gonimoblast in their figure is wide, thick-walled, septate, and branched, a condition the present investigation has shown to be indicative of a late stage in development. It seems likely, therefore, that Thuret and Bornet did not see the earliest stages and interpreted the secondary fusions incorrectly, supposing them to have preceded the development of the gonimoblast.

During the development of the carposporophyte the auxiliary cell branch shows characteristic changes, particularly concerning the branching and number of nuclei per cell. As soon as a gonimoblast fuses with a nutritive auxiliary cell, the latter increases in size, usually becoming spherical or ovoid and its cytoplasm showing greater affinity for Brazilin and haematoxylin. The nucleus and pit-connexions also increase in size. Frequently the nucleus divides and gives rise to two, three, or four nuclei. Examples have been observed in which the auxiliary cell nucleus had divided into four daughter nuclei, while the gonimolobe-initial was still uninucleate, and others in which the auxiliary cell showed a single enlarged nucleus at a very advanced stage of gonimolobe development.

Other cells of the auxiliary cell branch, especially the two or three cells lying immediately below and above the nutritive auxiliary cell, show changes similar to those that take place in the auxiliary cell fusing with the gonimoblast.

The large deeply stained uni- or multi-nucleate auxiliary cells with two or three neighbouring multi-nucleate cells with asymmetrical protrusions are



TEXT-FIG. 4. A and B. Secondary fusions of the gonimoblast with cells of the fertile branch. In A the gonimoblast has produced a downward growth which has fused with the third and fourth cells and another branch has arisen from the downward growth. Nucleus of gonimoblast with dark nucleolus and nuclei of gametophyte with white nucleoli. B. Fusions have taken place with the third and fifth cells of the fertile branch. The dilated cell is the stalk-cell of gonimolobe from which the cells have been severed. Most of the cells of the fertile branch are binucleate. Nuclei of gonimoblast indicated by dark nucleoli. C. Auxiliary cell branch from an old nemathecium showing the enlarged stalk-cell. Auxiliary cell four-nucleate and the cell below two-nucleate.

c, central cell; *cp*, carpogonium; *gbl*, gonimoblast; *st*, stalk-cell.

(A, Brazilin; B and C, iodine.) A ×566; B ×166; C ×285.

conspicuous in stained squashes and sections (Pl. VI, Fig. 12). In old nemathecia containing mature carposporangia, the cells of auxiliary cell branches are larger than those of the neighbouring filaments and their pit-connexions are conspicuous, sometimes occupying the breadth of the septum. In stained sections of nemathecia such branches can easily be traced to the centre of the thallus. This fact indicates that the food materials pass to the gonimolobes via the nutritive auxiliary cell. In old auxiliary cells the region of contact with the gonimoblast becomes slightly elongated and in some instances the greatly enlarged stalk-cell of the gonimolobe has the appearance of a side process of the auxiliary cell (Text-fig. 4, C).

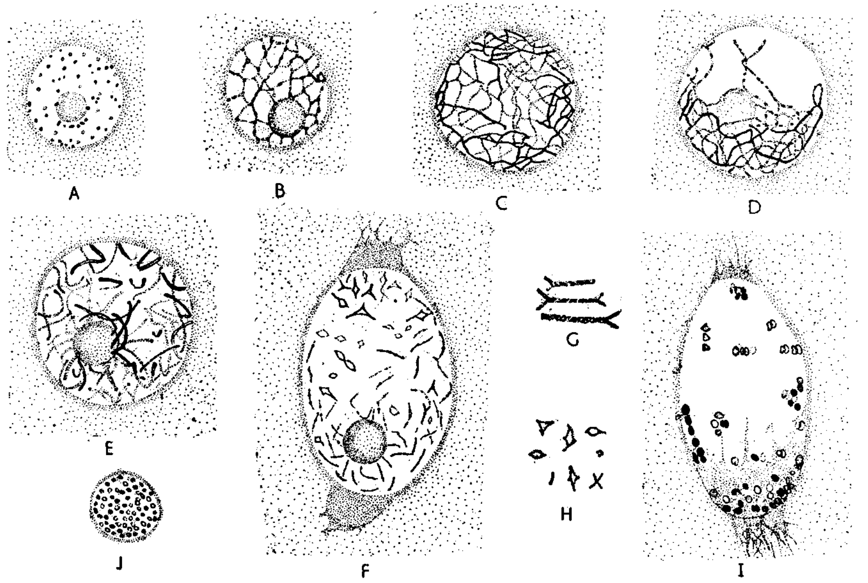
5. Reduction Division in the Tetrasporangium and other Cytological Observations

The following account is based on squash preparations stained with either Brazilin or Feulgen's reagent. For revealing the details of the structure of chromosomes in the early stages, Brazilin preparations were found to equal and sometimes even excel the corresponding Feulgen preparations.

Tetrasporangium-initials which arise from the cells of the inner cortex can be recognized by their dense cytoplasm and comparatively large nuclei. The sporangium-initial grows, ultimately reaching its full size, accumulating more and more food, particularly floridean starch. Likewise the nucleus also increases in size and passes through stages which bear a close resemblance to those described by Drew (1934) in *Spermothamnion turneri.* At first the nucleus is near the centre of the sporangium, but during the later prophase stages it lies to one side. In the earliest stages the nucleus has a nuclear membrane and a conspicuous nucleolus, surrounded by a number of small deeply staining bodies or granules. Although these granules appear as distinct entities in both Brazilin and Feulgen preparations, they cannot be counted at this stage as they are numerous and are very close together (Text-fig. 5, A). It has been found impossible to decide whether or not these granules are interconnected, but they are in all probability the heterochromatic regions of chromosomes. In a slightly more advanced stage (Text-fig. 5, B) the granules are definitely connected by less deeply staining threads. Gradually these slender threads take on the appearance of an intricate net-work uniformly distributed within the nucleus (leptotene) (Text-fig. 5, C). In the following stage the threads contract to one side of the nucleus, usually the basal one. Careful observation of the extremities of the threads reveals that they are made up of alternately stained and unstained regions (Text-fig. 5, D and Pl. I, Fig. 2). This appears to be the zygotene stage which, however, is probably of a short duration, the number of sporangia with nuclei at this stage being small.

In the next stage the 'U'- or 'V'-shaped threads are distributed throughout the nuclear area (Text-fig. 5, E and Pl. I, Fig. 3). They appear thicker and more homogeneous than during the preceding stages and this probably represents pachytene. From now onwards until late diplotene the threads do not stain readily and represent the 'diffuse stage' of Westbrook (1928, 1935). The threads become short and straight and lie near the periphery of the nucleus. In favourable Feulgen preparations some of them have the appearance of two threads coiled around each other at one or both ends (Text-fig. 5, G), probably indicating the double nature of the threads. As the nucleus and nucleolus enlarge further, the bivalents become more slender and are studded at intervals with thicker regions. Some of the bivalents are short and straight, while others are long and bent.

The nucleus and nucleolus attain their maximum size in the diplotene stage. The outline of the nucleus is elliptical in section, its long axis being parallel to the long axis of the tetrasporangium. Numerous bivalents can be seen distributed all over the nuclear area (Text-fig. 5, F). The doubleness of the bivalents manifests itself very clearly as the homologous chromosomes, having presumably become duplicated by now, loose their mutual attraction but are held together by the chiasmata. The bivalents assume various shapes and sizes (Text-fig. 5, H), most of them having only one chiasma and appear rhomboidal or as X's when unterminalized and as rods when terminalized.



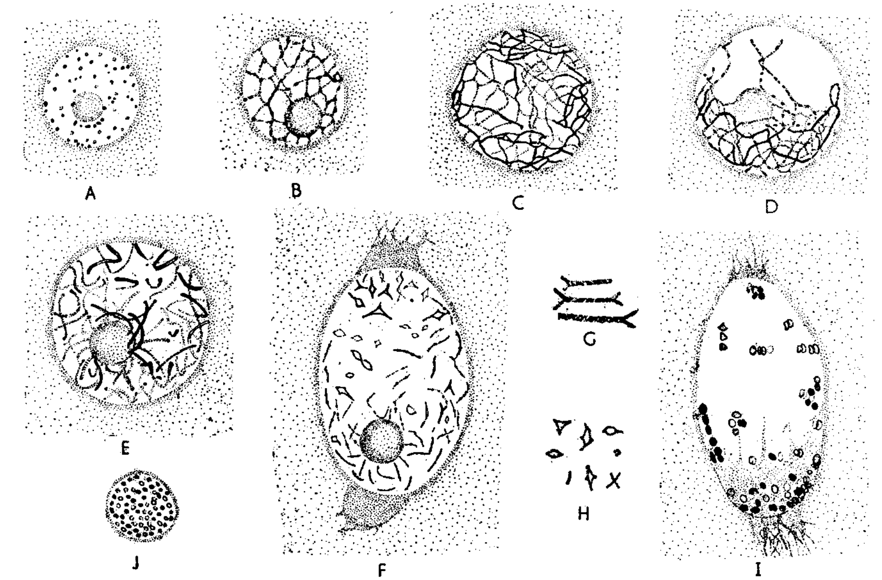
TEXT-FIG. 5. A–I. Meiosis in the tetrasporangium. A. Early prophase nucleus showing heterochromatic regions of the chromosomes. B. Darkly staining bodies interconnected by lightly stained regions. C. Slender chromatin threads uniformly distributed throughout the nucleus (leptotene). D. Chromatin contracted towards one side (zygotene). E. U- and V-shaped threads uniformly distributed (pachytene). F. Early diplotene nucleus. Note the large side of the nucleus and nucleolus, the numerous bivalents showing chiasmata and the conspicuous cytoplasmic areas at either pole of the nucleus. G. Twisted chromosomes with forked ends from nucleus shown in Pl. I, Fig. 4. H. Bivalents from a Feulgen preparation. I. Diakinesis. 68 bivalents could be counted. Note the distribution of the bivalents in groups and the cytoplasmic areas at either pole. J. Prophase nucleus of apical cell of a female plant. 65–68 chromosomes could be counted in this nucleus.

(A, B, D, E, and F Brazilin; the rest Feulgen.) ×1665.

Some bivalents have two chiasmata and look like rings, while occasional bivalents have three chiasmata resembling the figure 8. It has often been observed that some of the bivalents are triangular. They are probably bivalents with a single chiasma in which one arm is lying parallel to the line of observation.

During diakinesis the bivalents assume a variety of shapes and sizes. In spite of their great contraction and generally round shape, the individual components of bivalents can sometimes be distinguished, now entirely separate from each other. Counts of the number present, i.e. the haploid number, have ranged from 68–72 (Text-fig. 5, I and Pl. I, Figs. 5 and 6).

seen distributed all over the nuclear area (Text-fig. 5, F). The doubleness of the bivalents manifests itself very clearly as the homologous chromosomes, having presumably become duplicated by now, loose their mutual attraction but are held together by the chiasmata. The bivalents assume various shapes and sizes (Text-fig. 5, H), most of them having only one chiasma and appear rhomboidal or as X's when unterminalized and as rods when terminalized.



TEXT-FIG. 5. A–I. Meiosis in the tetrasporangium. A. Early prophase nucleus showing heterochromatic regions of the chromosomes. B. Darkly staining bodies interconnected by lightly stained regions. C. Slender chromatin threads uniformly distributed throughout the nucleus (leptotene). D. Chromatin contracted towards one side (zygotene). E. U- and V-shaped threads uniformly distributed (pachytene). F. Early diplotene nucleus. Note the large side of the nucleus and nucleolus, the numerous bivalents showing chiasmata and the conspicuous cytoplasmic areas at either pole of the nucleus. G. Twisted chromosomes with forked ends from nucleus shown in Pl. I, Fig. 4. H. Bivalents from a Feulgen preparation. I. Diakinesis. 68 bivalents could be counted. Note the distribution of the bivalents in groups and the cytoplasmic areas at either pole. J. Prophase nucleus of apical cell of a female plant. 65–68 chromosomes could be counted in this nucleus.

(A, B, D, E, and F Brazilin; the rest Feulgen.) ×1665.

Some bivalents have two chiasmata and look like rings, while occasional bivalents have three chiasmata resembling the figure 8. It has often been observed that some of the bivalents are triangular. They are probably bivalents with a single chiasma in which one arm is lying parallel to the line of observation.

During diakinesis the bivalents assume a variety of shapes and sizes. In spite of their great contraction and generally round shape, the individual components of bivalents can sometimes be distinguished, now entirely separate from each other. Counts of the number present, i.e. the haploid number, have ranged from 68–72 (Text-fig. 5, I and Pl. I, Figs. 5 and 6).

The nucleolus stains well with Brazilin up to diplotene or diakinesis, but subsequently it does not appear as homogenous and shining. It becomes opaque and lobed as the prometaphase stage approaches. Ultimately it degenerates and forms a cloudy mass around the bivalents. Neither vacuolation nor disintegration of the nucleolus into separate pieces has been observed.

When all the bivalents ultimately occupy an equatorial position they are crowded together and their individual identity can no longer be made out. This appearance at metaphase has been a very constant feature in all preparations irrespective of the fixatives employed, but should probably not be considered normal. As a result of this, disjunction cannot be observed, but presumably takes place and the two sets of chromosomes move apart as compact plates. The surrounding cytoplasm becomes denser, and as the plates move apart they are surrounded by irregular masses of densely staining cytoplasm.

A wall, the formation of which starts during metaphase, separates the protoplast into two uninucleate cells and thus a diad is formed before the nucleus undergoes the second division. Since such diads are frequently met with, it can be inferred that the interphase between the first and second divisions is one of long duration. Subsequently, each cell divides into two and thus four spores are formed in a tetrasporangium.

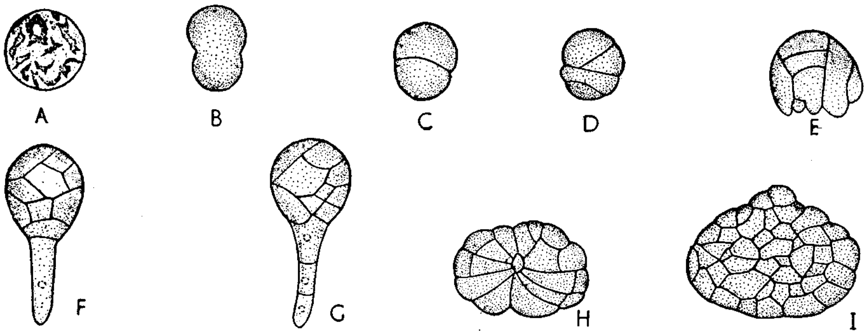
Late prophase stages in mitosis have been seen in large numbers in various types of cells such as the apical cells and medullary filaments of sexual and tetrasporic plants, gonimolobes, and spermatangial filaments, but critical counts of chromosomes from these cells could not be obtained due to the large number of chromosomes in relatively small nuclei. A few counts of the chromosomes found have, however, been obtained from the apical cells and medullary filaments of the main axis and apical cells of sterile nemathecial filaments of female plants. Text-fig. 5, J and Pl. VI, Fig. 9 show a prophase nucleus from the apical cell of a female plant in which approximately 65–68 chromosomes have been counted. It has not been possible to obtain accurate chromosome counts of tetrasporic individuals, but the number is sufficiently large to indicate that it is double the haploid number. Pl. I, Fig. 10 shows the nucleus of a medullary filament of a tetrasporic plant in which up to 130 chromosomes have been counted.

6. Early Stages in the Germination of Spores

The culture solution and tank used for germinating tetraspores and carpospores and also the culture tank are the same as those employed by Drew (1952). Crystalline penicillin has proved very useful in clearing cultures of germlings of bacteria. A concentration of 10 units of penicillin per 1 c.c. of culture solution (cf. King, 1950) has been used for this purpose.

Tetraspores have been germinated on seven different occasions during the period of this investigation. Sporelings germinated during November 1952 were kept growing for about 13 months, when they were abandoned as growth became very slow and they were heavily overgrown by diatoms and filamentous algae.

When sections of fertile regions of tetrasporic plants are floated in culture solution contained in petri-dishes the liberated spores, being heavier than water, sink to the bottom. They enlarge and become spherical. The first division in such spores may occur after varying intervals of time ranging from a few days to a month. In one instance, healthy undivided spores could be seen at the bottom of the culture dish 2 months after liberation. The first wall may appear in a spore after it has elongated slightly or without any change of shape. Either one or both the resulting cells participate in subsequent divisions although one of the cells may simply elongate into a septate or aseptate rhizoid (Text-fig. 6, F and G). Subsequent divisions of the spore



TEXT-FIG. 6. Early stages in the germination of tetraspores. × 200.

follow no regular pattern but ultimately a multicellular hemispherical body results. Sometimes several peripheral cells on one side elongate to form rhizoidal outgrowths, which either persist or degenerate again. After a time the rate of growth becomes slow, but small purple-red upright shoots appeared, however, after 4 months on a few of these germlings. Each disk may bear one to three such upright axes. Although a plant was maintained for 13 months, there was no formation of any reproductive organs. The stages in the germination of a carpospore closely resemble those of a tetraspore.

Thuret and Bornet (1878) state that they did not succeeed in germinating tetraspores, although they obtained multi-cellular cushions from germinated carpospores. The irregular divisions of the tetraspores and carpospores recall the germination stages of certain Cryptonemiales and Gigartinales figured by Chemin (1937).

7. DISCUSSION[1]

This investigation of the development of the reproductive organs, the carposporophyte, and the life-history of *P. caprinus* has revealed features of interest which will now be discussed.

[1] The views expressed here regarding nemathecia and the interpretation of the filaments bearing carpogonia have been arrived at after discussion with Dr. K. M. Drew, who also supports them.

The tetrasporangia of *P. caprinus* develop in unspecialized upper portions of the thallus, endophytes often occupying the cavities left by the dispersal of the spores. In contrast, the sexual reproductive organs develop in special superficial mound-like growths referred to as nemathecia (Kylin, 1923; Taylor, 1937; Fritsch, 1945; Drew, 1951). Long ago, Greville (1824) called them 'spongioles' and Thuret and Bornet (1878) 'plaques'. This restriction of the sexual organs and carposporophyte to such superficial areas—which are later shed—is probably of considerable biological advantage to a perennial species such as *P. caprinus*.

Fritsch (1945) reports that nemathecia occur in the families Rhizophyllidaceae, Squamariaceae, Gigartinaceae, and Phyllophoraceae, but closer consideration shows that the structures in question are certainly not homologous. The term nemathecium appears to have been used by Smith (1938) and Fritsch (1945) to signify any outgrowth of a given appearance irrespective of origin, although Phillips (1925) tries to be more precise than others by restricting its usage to the warty outgrowths bearing tetrasporangia. A clear distinction must be made between structures which arise entirely from the gametophyte (*P. caprinus*) or tetrasporophyte (*Peyssonnelia dubyi*) and contain reproductive organs and those which develop from a generative auxiliary cell and are therefore solely the carposporophyte (*Phyllophora brodiaei*). Among those algae in which the sexual reproductive organs are found in special outgrowths of the gametophyte, it is found that there are two types; firstly, those in which the outgrowth shows the same structure as the main thallus bearing it, as in *Gigartina stellata* (Drew and Greig-Smith, in Newton, 1949), and secondly, those in which the outgrowth is different in structure from the main thallus, e.g. *P. caprinus*. It is to the latter that it would seem best to restrict the use of the term nemathecium, a term first used by C. Agardh (1822) for the wart-like outgrowths found on *Ahnfeltia plicata*. As the significance of these is still doubtful, a fresh definition is much needed and it is suggested that the term *nemathecium is used for a mound-like growth of specialized filaments produced by the further growth of the superficial cells of the gametophyte or tetrasporophyte and bearing the appropriate reproductive bodies.* Where carpogonia occur in the nemathecia, the resultant carposporophytes will in all probability develop inside the nemathecia also.

The carpogonia of *P. caprinus* have been shown to be terminal on certain filaments of the nemathecium. These nemathecial filaments may be interpreted either as fertile branches bearing sessile carpogonia or very long carpogonial branches. The former interpretation has been accepted in this account and the branches in question have been referred to as fertile branches. The reasons for this view are, firstly, the filaments in question are continuations of outer cortical filaments, and secondly, the cells of the filament terminated by a carpogonium are not necessarily any different from neighbouring sterile filaments at the time of fertilization. Many instances have been observed in which the fertilized carpogonium had formed a gonimoblast without any of the subtending cells showing any change of contents. On the basis of this interpretation of the filament as a fertile branch the carpogonia are sessile. Sessile carpogonia have been reported previously in several members of the Rhodophyta from various orders (e.g. in *Petrocelis hennedyi* and *Cruoria pellita*, Rosenvinge, 1917; in *Rhodochorton violaceum*, Drew, 1935; and in *Gelidium cartilagineum*, Kylin, 1923). In view of these records, it appears likely that sessile carpogonia may be more common in the Rhodophyta than hitherto reported.

There are at present no well-defined criteria for deciding when a carpogonium is mature, but it is generally assumed to coincide with the stage when its trichogyne attains its greatest length. In *P. caprinus* it has been observed that it is the first-formed carpogonia with short trichogynes which are fertilized and that it is likely that carpogonia with very long trichogynes are not. Possibly the trichogynes of unfertilized carpogonia elongate indefinitely, keeping pace with growing sterile nemathecial filaments and ensuring the exposure of the terminal receptive region. In old nemathecia numerous such unfertilized carpogonia with greatly elongated filiform trichogynes are always seen.

There are several features of the organization of this carposporophyte which call for comment. It has been shown that a single branched gonimoblast develops from the carpogonium direct, and that although this fuses with certain cells of the nemathecial filaments, there is no nuclear migration from the gonimoblast to these cells. For this reason and the fact that the carpospore-producing branches, the gonimolobes, develop from the gonimoblast, these cells of the nemathecial filaments are considered to be nutritive auxiliary cells as defined by Drew (1954). It has also been shown that the gonimolobes may arise in various positions and not necessarily in close proximity to the nutritive auxiliary cells. In addition, they outnumber the auxiliary cells. These observations provide additional evidence in support of the views put forward by Drew (1954) that the type of carposporophyte exemplified by *Dudresnaya coccinea*, in which the gonimolobe develops from the auxiliary cell after a transfer of a nucleus from the gonimoblast, has arisen from the type, illustrated by *Sirodotia* or *Dermonema*. In these the spreading gonimoblasts do not fuse with cells of the gametophyte and thus *P. caprinus* is an intermediate type, the gonimoblast fusing with cells of the gametophyte, the nutritive auxiliary cells, but there is no transfer of nuclei and the gonimolobe formation takes place on the gonimoblast, Drew's Group B, in fact.

Evidence has been given for considering the fusions of the gonimoblast with cells of the fertile branch as secondary. They do not appear to be obligatory judging from the small number of examples observed relative to the amount of material examined, and thus differ from those which occur in the Dumontiaceae. It is possible that the fusions are of a completely different category altogether.

The cytological evidence indicates that reduction division takes place in the tetrasporangium of *P. caprinus*. The very high haploid number of *c.* 70 has made accurate counts difficult. There is evidence that the number of bivalents counted at diakinesis (68–72) roughly corresponds with the haploid chromosome counts obtained from the apical cells of sexual plants. Although counts have not been possible a considerably large number of chromosomes has also been seen in gonimolobes and the medullary cells of tetrasporic plants. It seems reasonable to conclude, therefore, that the life-history of this alga in the British Isles is of the *Polysiphonia*-type, i.e. it is cytologically diphasic (Drew, 1944).

An extended investigation would show whether the same would be true for other localities such as Denmark and Sweden, where the available information, showing that sexual thalli predominate, suggests it might not be. The high numbers of chromosomes, whatever the signficance, has no parallel in the investigated Florideae.

In conclusion I wish to express my grateful thanks to Dr. K. M. Drew for her guidance, encouragement, and criticism throughout the course of the investigation and help in the preparation of this paper, and to Professor C. W. Wardlaw for his encouragement and advice. I should like to thank all those who assisted me with the collection of material, especially Dr. M. T. Martin, Dr. S. M. Lodge, and Mr. H. T. Powell. My thanks are also due to Mr. E. Ashby for the photographic illustrations.

I wish to take this opportunity of thanking the authorities of the Banaras Hindu University for enabling me to study in Manchester.

8. Summary

1. Sexual organs develop in superficial nemathecia which arise in a fortuitous manner on the upper dichotomies of male and female plants Spermatangial filaments are richly branched and brush-like in appearance. Carpogonia are terminal and sessile on undifferentiated fertile branches.

2. Undifferentiated intermediate cells of nemathecial filaments rich in floridean starch and situated at varying distances from the carpogonium, function as nutritive auxiliary cells. They cannot be distinguished until a gonimoblast fuses with them. There is no transfer of the diploid nucleus into the auxiliary cell or any cell of the gametophyte.

3. A single gonimoblast develops direct from the fertilized carpogonium without any prior fusions with the cells of the fertile branch. It branches profusely, fusing with a number of nutritive auxiliary cells and in the early stages bears gonimolobes near the regions of fusion with these cells. During the last stages in its development gonimolobes may be formed on the gonimoblast remote from auxiliary cells.

4. Secondary fusions between the cells of the fertile branch and the base of the gonimoblast have been seen in twelve instances and occur at a late stage in the development of the carposporophyte.

5. Cruciate tetrasporangia develop from cells of the inner cortex of special plants. Reduction division precedes tetraspore-formation. The haploid

chromosome number of 68–72 established from counts at diakinesis is very considerably higher than any previously reported in the Florideae.

Nuclei have been seen in division in apical cells of the thallus, gonimoblast, and spermatangial filaments, but the large number of chromosomes has precluded accurate counts. The cytological evidence suggests, however, that the life-history in the British Isles is of the *Polysiphonia*-type.

6. The germination of tetraspores and carpospores is alike. After some irregular cell-divisions a hemispherical cushion of cells results. Growth of sporelings in culture is very slow, and upright axes arise from the disks only after several months.

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EXPLANATION OF PLATE VI

Illustrating C. S. Prakasa Rao's article on 'The Life-History and Reproduction of *Polyides caprinus* (Gunn.) Papenf.'

Fig. 1. Nucleus of tetrasporangium showing uniform distribution of chromatin. Late leptotene. ×2000.

Fig. 2. Nucleus of tetrasporangium showing contraction of chromatin to one side. Alternate stained and unstained regions visible in the threads. Zygotene. ×2000.

Fig. 3. U- and V-shaped thicker threads showing the stained and unstained regions more clearly. Pachytene. ×2000.

Fig. 4. Straight threads with forked ends. Late pachytene. ×2000.

Fig. 5. Diakinesis. 68 bivalents could be counted in this nucleus. Composite drawing of this nucleus given in Text-fig. 5, I. ×2000.

Fig. 6. Diakinesis. 72 bivalents could be counted in this nucleus. ×2000.

Fig. 7. Part of a transverse section of a very young nemathecium. Note the deeply stained male nucleus within the trichogyne. Very little of base of carpogonium in this section. ×1000.

Fig. 8. Fusion of male and female nuclei within the carpogonium. Note the constricted neck of the trichogyne. The dark areas in the trichogyne are the nuclei of cells behind the trichogyne. ×2000.

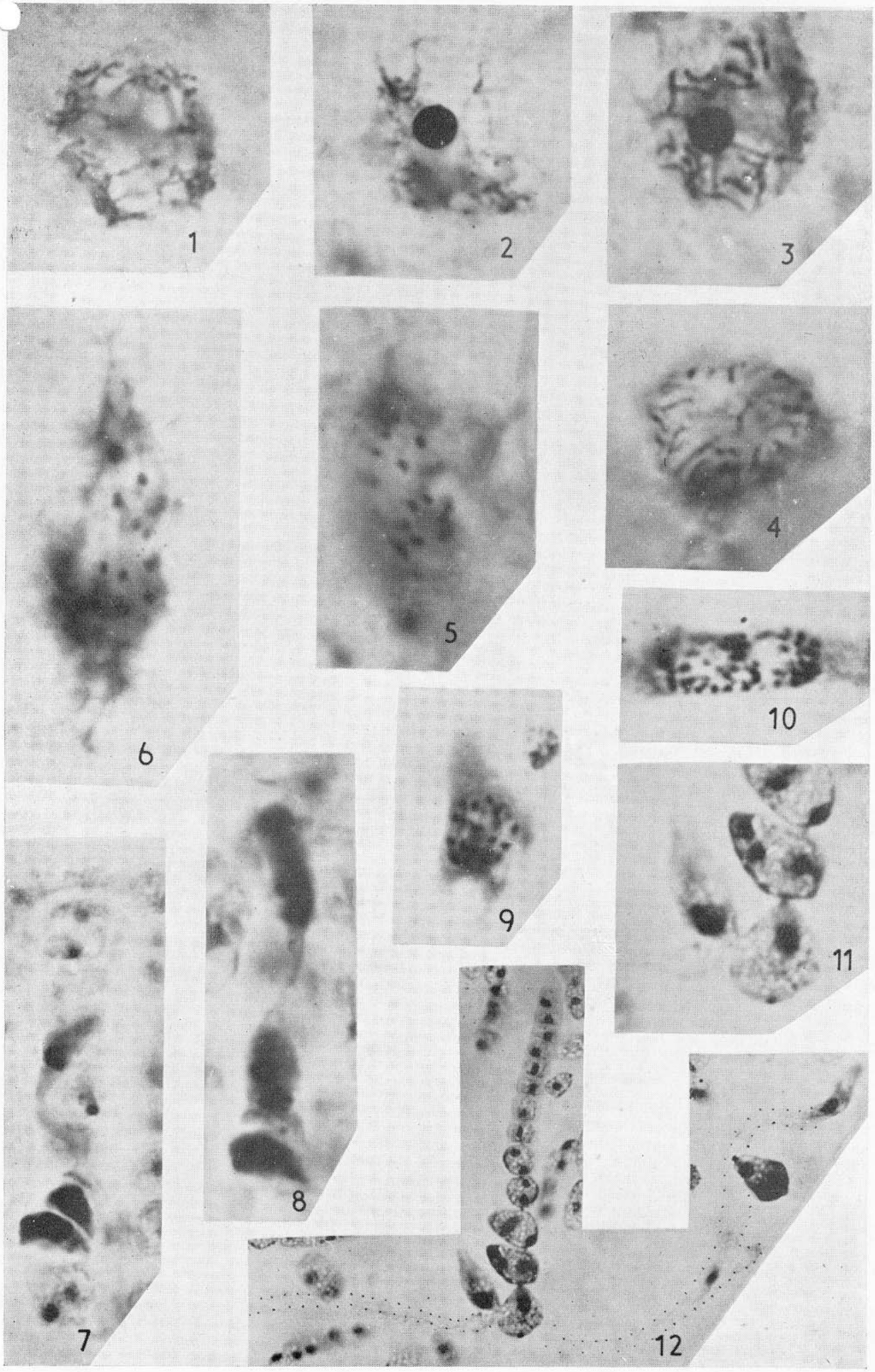
Fig. 9. Nucleus of apical cell of female plant. 65–68 chromosomes could be counted in this nucleus. Composite figure of this nucleus shown in Text-fig. 5, J. ×2000.

Fig. 10. Nucleus of a medullary filament of a tetrasporic plant. Up to 130 chromosomes could be counted in this nucleus. ×2000.

Fig. 11. Formation of gonimolobe-initial as an outgrowth from the expanded region of contact between the gonimoblast and the nutritive auxiliary cell. Auxiliary cell uninucleate and two cells above multinucleate. ×1000.

Fig. 12. More general view of the auxiliary cell branch of Fig. 11 and the gonimoblast. The latter is very lightly stained and its outline is emphasized with ink. Note the prominent nucleus in its tip. ×500.

Figs. 1, 4–6, 9, 10 Feulgen.
Figs. 2, 3, 7, 8, 11, 12 Brazilin.



C. S. P. RAO