POLYMORPHISM, SEXUAL REPRODUCTION, MEIOSIS AND POLYPLOIDY AND THEIR IMPLICATIONS FOR DESMID TAXONOMY

by

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I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and that, to the best of my knowledge and belief, the thesis contains no copy or paraphrase of material previously published or written by another person, except when due reference is made in the text.

Huang

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CONTENTS

ABSTRACT

Conjugation and zygospore germination are described for 6 *Pleurotaenium*, 2 *Micrasterias* and 1 *Staurastrum* species. Probable chemotaxis is demonstrated in one *Pleurotaenium* species and selective mating, where a plus clone actively chooses one of two minus clones, is shown to be common in the *P. mamillatum* complex.

The *Pleurotaenium* species show remarkable homogeneity in conjugation features, possession of 5-walled smooth zygospores with operculate exospores and mammillate mesospores and producing single germination products. Zygospores of *M. hardyi*, *M. laticeps* and *St. orbiculare* var. *ralfsii* are spiny, 3-walled, but only the *Micrasterias* zygospores are operculate. All three produce 2 germination products.

Meiosis is described in germinating zygospores from crosses between various karyotypes and ploidy levels of the *P. mamillatum* complex, between haploids and diploids and morphologically different strains for other species of *Pleurotaenium* and the two *Micrasterias* species. In the *Pleurotaenium* species meiosis is chiasmate, conventional in character and the chromosomes have localized centromeres. Sexual crossing, resulting in viable, fertile offspring, occurs to a surprising degree between strains with widely disparate chromosome numbers, ploidy levels and geographical location. These strains form discrete groups that are reproductively isolated from one another.

The implications that the results have for desmid taxonomy are discussed.

I. INTRODUCTION

In the course of investigating the limnology of Arthurs lake, Tasmania, it was found necessary to identify the desmids which form an important and major component of the lake's phytoplankton. This proved extremely difficult as, frequently, not only was it possible to identify a single desmid with a number of species but also, in several cases, the desmid could, quite legitimately, be assigned to two genera, and even three in one instance.

It is evident, from the literature, that desmid taxonomy is based almost entirely on morphological characteristics of vegetative cells, with little emphasis on seasonal and environmental variation, and almost total disregard of sexual characteristics. This stems largely from the typological approach towards classification of most algae. Phycologists have rarely given consideration to the concept of a biological species (sensu Mayr, 1963). However, recent studies involving breeding patterns in the *Volvocales* (Coleman, 1959 and in press; Stein, 1965) and especially the Charophyta (Proctor, 1975) have done much to clarify the taxonomy and our understanding of these groups.

Desmidiologists, until recently, have concerned themselves primarily with floristic surveys and for the most part alpha-level taxonomy. Few have undertaken intensive cultural studies or the use of sexual reproduction as an aid in recognising species. This is mainly because of the rarity or even the absence of sexual reproduction among certain groups, the high percentage of homothallism and a general misconception that desmids either were difficult to culture

or lack sexual potential. Nevertheless, over the past 20 years, studies by Starr (1954, 1959), Cook (1963), Biebel (1964), Lippert (1967), Gerrath (1969, 1970, 1973) and Pickett-Heaps (1975) have demonstrated the feasibility of various experimental approaches including crosses between heterothallic strains, ultrastructural studies and electron microscopy. However, the studies on sexual reproduction were, for the most part, concerned with descriptions of the sexual process itself, detailing methods of inducing conjugation, the conjugation process and zygospore germination (though work by Starr (1959) and Brandham & Godward (1965b) did indicate the compatibility of *Cosmarium* populations from relatively close but separate geographical areas) and the possibility of multiple mating pairs. Cook's (1963) investigations revealed that features of sexual reproduction in species of *Closterium* are more permanent and discrete than vegetative characters. He proposed that sexual morphology be an essential feature in the identification and description of taxa.

To date there has been little desmid research along the lines that Proctor (1975) used for the Charophyta, and the taxa of desmids remain tenuous, species grading into species, genera merging into one another.

In an attempt to clarify the taxonomic problems of the Arthurs Lake desmids, several species were isolated into culture. Only three species could be induced to produce zygospores. Under the assumption that most of the species may actually represent single clonal

populations (Teiling, 1950; Brandham & Godward, 1965b) collections were made from other Tasmanian localities and from the Australian mainland, primarily seeking sexual clones but also with a view towards testing sexual compatibility between geographically isolated populations. Several more sexual clones, predominantly *Pleurotaenium* species, were uncovered. A detailed study of sexual reproduction in two *Pleurotaenium* species (Ling & Tyler, 1972a, 1972b) revealed the distinct possibility that at long last we may have a genus that is sharply delineated from all other desmid genera. Certain aspects of the *Pleurotaenium* life-cycle, such as a pre-secreted conjugation vesicle and the single gonal product, were unusual. The outstanding observations were two features of the zygospore, a morphologically differentiated operculum and a mammillate mesospore. It was suggested that these two features may prove to be useful generic criteria of *Pleurotaenium*. Subsequently, to confound matters, an operculum was found in the *Micrasterias laticeps* zygospore, precipitating research on sexual reproduction in several other *Pleurotaenium* species, *M. hardyi* and other desmids.

Desmids are normally haploid organisms in the vegetative phase, the only diploid phase being the zygospore. The haploid form is restored through meiosis when the zygospore germinates. As yet our understanding of the meiotic process in desmids is inadequate. Descriptions of meiosis in desmids are rare. The reports of Klebahn (1890), Pothoff (1927), Starr (1954, 1955b), Fox (1958) and Biebel (1964), covering the genera *Cosmarium, Closterium, Hyalotheca* and

Netrium, were sketchy. The only detailed report, covering the mechanism of the meiotic process in two varieties of *Cosmarium botrytis*, is that of Brandham and Godward (1965a).

The desmids present a fascinating and vast range of organisms whose potential for the study of physiology and morphogenesis has been largely unexploited. Being unicellular and morphologically diverse they offer distinct advantages when compared to multicellular plants, especially in the possibility of following the development of a solitary cell under the microscope. They are also worthy of study and culturing purely from an aesthetic point of view.

II. AIMS

1. To isolate sexual strains of desmids for the following purposes:
   (i) to create sound generic delineation for *Pleurotaenium* based on sexual criteria,
   (ii) to ascertain whether sexual characters in other desmid genera are useful generic criteria,
   (iii) to distinguish between environmental, genotypic and species differences in morphology,
   (iv) to establish variant strains, from natural collections or through selective breeding, and use the differences as identifying tags,
   (v) to test the sexual compatibility of strains from different localities,
   (vi) to study the process of meiosis and obtain chromosome counts.

2. To reinvestigate the alpha taxonomy of the *Pleurotaenium mamillatum* complex.

In the course of these investigations many other intriguing facets of desmid behaviour and development were encountered. These include selective mating behaviour and the correlation of the number of gonal products with the number of chromatophores in the germinating zygospore. The results are also incorporated.

III. MATERIALS AND METHODS

Collection.

Samples were collected from ponds, swamps and lakes with a 20 μm plankton net.

Isolation.

A sample was poured into a watch-glass. With a micro-pipette, selected cells were transferred to a plate of desmid agar. Using a glass needle pulled to a fine point, a cell was manoeuvred across the agar until free of adhering particles. The cell, on a minute agar block, was then transferred to a fresh plate or a culture tube.

Media.

(a). Desmid agar (Waris, 1953; Starr, 1964).

To distilled water was added 10 ml of each of (i) to (iii), 5 ml of each of (iv) and (v), 50 ml of soil extract, the solution made up to 1 litre, and solidified with 7.5 gm of agar.

(i). 1.0% solution of KNO_3.

(ii). 0.15% solution of $MgSO_4.7H_2O$.

(iii). 0.25% solution of $CaSO_4.2H_2O$.

(iv). 0.05% solution of K_2HPO_4.

(v). 0.1% solution of $(NH_4)_2HPO_4$.

Soil extract was prepared by autoclaving 1 kgm of sandy loam with 1 litre of distilled water for 15 minutes. The cooled supernatant was then centrifuged at 9000 rpm for 30 minutes to remove suspended particles.

(b). Soil-water medium (Starr, 1964).

Soil-water medium was made by placing,

(i) 1 cm layer of loam in the bottom of a 1.8 cm x 15 cm test tube and then adding distilled water till 2/3 full, or

(ii) ½ cm layer of loam in the bottom of a 250 ml conical flask and then adding 200 ml of distilled water.

The tubes or flasks were then plugged with cotton and steamed for 1 hour on two consecutive days.

Loam.

Samples of sandy loam were collected from various places. Mixtures that supported good growth of most desmids were then made up and used for soil extracts and soil-water media. The following two mixes were commonly used for soil-water media. S_{46} contained soil samples S_4 and and S_6 in the ratio 3 : 1. S_{14R} contained soil samples S_1, S_4, S_R in the ratio 1 : 2 : 1.

S_1 - brown loam from Hobart Botanical Gardens.

S_4 - sandy bracken-loam from Risdon Vale.

S_6 - dark clayey soil from Hobart Botanical Gardens.

S_R - light-brown loam from Richmond.

Growth and Maintenance.

Cultures were grown and maintained at 16 - 22°C and illuminated by Gro-lux fluorescent tubes on a 16 - 8 hour light-dark cycle. The light intensity was about 2000 lux.

Induction of Conjugation.

Vigorously growing soil-water cultures were used for crossing. For *Micrasterias* and *Staurastrum*, conjugation was induced by mixing clones in watch-glasses at about 25°C under a 16 - 8 hour light-dark regime, using 5% Na HCO_3 as CO_2 source (Starr, 1955a).

Pleurotaenium clones were mixed a few hours before the onset of the light cycle. Enough inoculum was added to cover the bottom of a petri-dish to a depth of 2 - 4 mm. The mixture was then placed under conditions identical to those for vegetative growth.

Cytology.

1 - 2 month old zygospores were immersed in fresh media. Released germination vesicles were pipetted into a fixative of a 1 : 3 mixture of glacial acetic acid and 95% ethyl alcohol.

Initially iron-aceto-carmine was used for staining (King, 1960); later it was found that aceto-orcein (Darlington & La Cour, 1962) was more convenient.

After fixing, the vesicles were washed once with distilled water and pipetted onto a slide. A drop of aceto-orcein was added and heated to boiling for a few seconds. After cooling a coverslip was placed on. Squashing was applied through a layer of filter paper.

To make slides permanent the slides were inverted in a dish containing fixative till the coverslip detached itself. The slide and coverslip were then passed through two changes of alcohol and then mounted in Euparal.

Photography.

Photomicrographs were taken on Ilford FP4 film on a Carl Zeiss (Oberkochen) Photomicroscope II employing bright field and Nomarski differential interference contrast. Drawings were made using the same microscope or a Zeiss (Oberkochen) RA microscope each fitted with a camera lucida with internally reflected image, allowing binocular viewing while drawing.

IV. RESULTS

A. The Sexual Process In *Pleurotaenium*.

The processes of pairing, conjugation, zygospore maturation, germination, meiosis and subsequent development is, more or less, identical in all the *Pleurotaenium* species studied. Few, and only minor, differences were observed.

Pairing usually occurs within twenty four hours after mixing clones. The conjugants come to lie close together, parallel or at a slight angle, with their isthmuses juxtaposed. Multiple pairing is frequent. A common mucilage envelope is secreted followed by the secretion of a conjugation vesicle between the isthmuses. The isthmuses lengthen, the semicells remaining joined by a cylinder of thin new wall material. A circular escape pore is gradually dissolved in this central cylinder. Gametogamy ensues in the conjugation vesicle. The process has been described previously (Ling & Tyler, 1972a).

The mature zygospore is dark brown to black and has five walls. Outermost is a thin membranous exospore I, followed by a thick exospore II immediately underneath. The mesospore is brown and mammillate. There are two thin, transparent endospores, the inner one slightly thicker than the outer. (Ling & Tyler, 1972a, Fig. 21).

Part of exospore II is differentiated into an ellipsoidal to circular, convex plate. This operculum has a smooth rim but is otherwise identical to exospore II in structure and sculpturing (Ling & Tyler, 1972a, Figs. 22, 30).

At the onset of germination, the protoplast still enclosed by the two endosporia, ruptures the brown mesospore, detaches the operculum, and escapes as an undifferentiated vesicle (Fig. 1). The newly released vesicle is usually a pale brownish to bright yellow colour. Yellow globules, starch grains and bits of haematochrome occupy the periphery of a large, clear central vacuole. A single dark green chromatophore is usually visible.

The newly released germination vesicle contains a single nucleus, usually at diakinesis to early metaphase I. After meiosis only one daughter nucleus survives. The protoplast shrinks and constricts to form a single *Cosmarium*-like product. Divisions of this gone then produce new semicells with the characteristic shape of the species. A detailed description of germination has been given previously (Ling & Tyler, 1972b).

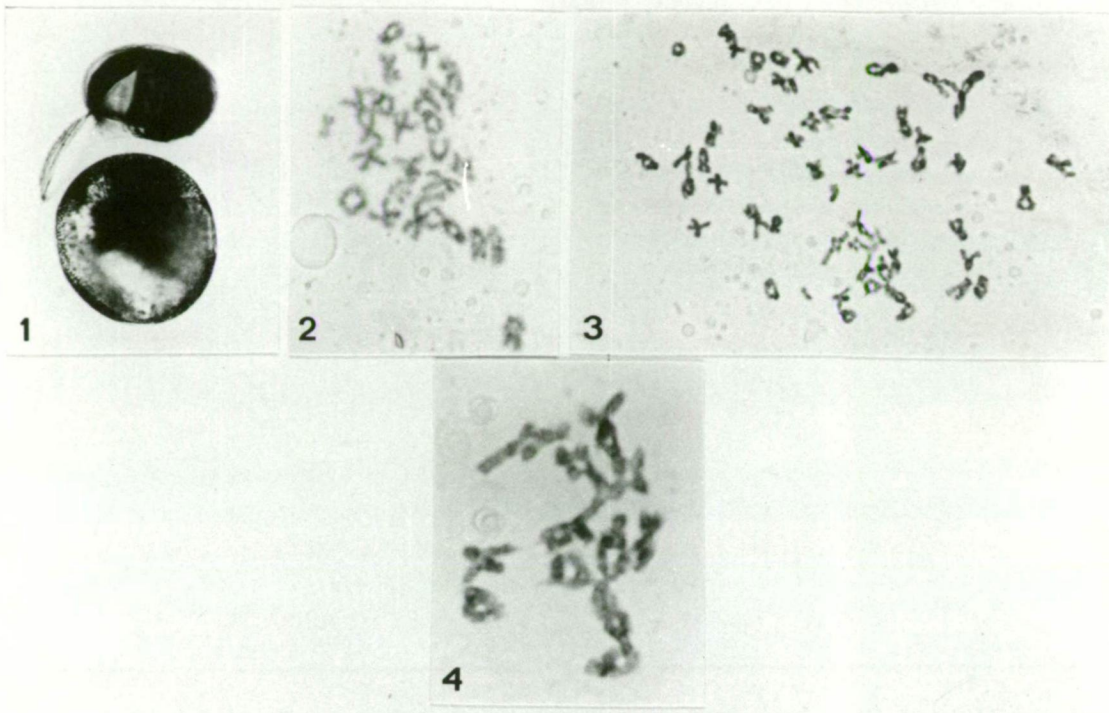

Fig. 1. Germination, showing newly released vesicle and operculum still hinged to exospore. x 200.

Figs. 2 - 4. Meiosis in *P. ehrenbergii*.

Fig. 2. Diplotene. Incomplete complement. Cross and ring bivalents are visible. x 1,000.

Fig. 3. Diakinesis, showing some stickiness between bivalents. x 790.

Fig. 4. Enlarged portion of Fig. 3. Top group shows sticky association between bivalents. x 2,000.

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1. The *Pleurotaenium mamillatum* Complex.

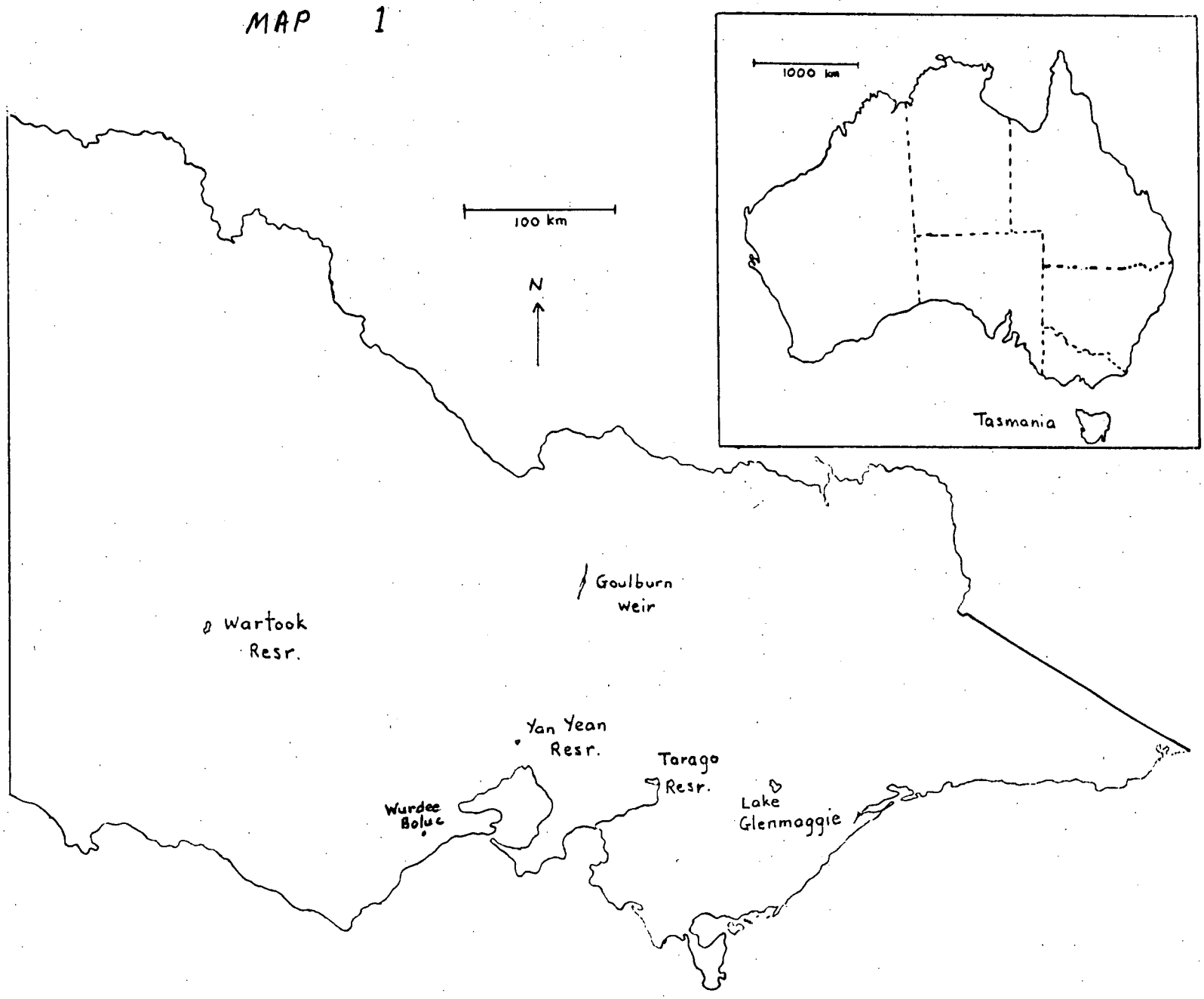
The complex contains three desmid taxa, isolated from different parts of Australia, conforming to the morphological taxa *Pleurotaenium ehrenbergii* (Breb.) de Bary, *Pleurotaenium mamillatum* G. S. West and *Pleurotaenium coronatum* (Breb.) Rabenh. The three taxa are interfertile to the extent of producing large numbers of zygospores. It was suggested that the three species are synonymous (Ling & Tyler, 1974). However, Bourrelly (personal communication) questioned the identity of the three species. Here the alpha taxonomy of these three *Pleurotaenium* spp. is reinvestigated and the cytogenetics of crosses between clones within and between the three morpho-species is described in detail. For reasons to be explained in the Discussion the three specific names are retained. The origin of strains is shown in Map 1 and Table 1. A total of eight *P. mamillatum* strains, all plus in mating type, were isolated from Yan Yean Reservoir. Only two are included in the table.

(i). Meiosis in *P. ehrenbergii* and *P. mamillatum*.

P. ehrenbergii.

Sexual reproduction in *P. ehrenbergii* (Ling & Tyler, 1972a, 1972b) follows the general process for *Pleurotaenium* spp.

Newly released germination vesicles contain a single nucleus, usually at diakinesis (Fig. 3) though diplotene stages are sometimes observed (Fig. 2), with fuzzy chromosomes which stain lightly with aceto-orcein. Chiasmata are clearly visible. Most of the bivalents have only one chiasma and assume a typical cross appearance. However, five to ten ring bivalents are usually present per cell.



Map of Australia (inset) and the state of Victoria showing the origin of strains of the *P. mamillatum* complex.

Table 1. Source and mating type of strains in the *P. mamillatum* complex.

Strain	Alpha species	Source	Mating type	Remarks
PEAL 1	*P. ehr.*	Arthurs L., Tas.	+	
PELS 1	*P. ehr.*	L. Sorell, Tas.	+	
PEWL 1	*P. ehr.*	Woods L., Tas.	+	
PEWL 2	*P. ehr.*	Woods L., Tas.	-	
PE F_1 1	*P. ehr.*	PEWL 2 x PELS 1	+	
PE F_1 2	*P. ehr.*	PEWL 2 x PEAL 1	'-' *	
PE F_1 3	*P. ehr.*	PEWL 2 x PEAL 1	+	Tapered
PE F_1 4	*P. ehr.*	PEWL 2 x PEWL 1	-	
PE F_1 5	*P. ehr.*	PEWL 2 x PEWL 1	+	
PE F_1 6	*P. ehr.*	PEWL 2 x PEWL 1	-	
PE F_1 7	*P. ehr.*	PEWL 2 x PEWL 1	+	
PEAL 1 2n	*P. ehr.*	Veg. derivative of PEAL 1 in culture	+	
PE F_1 3 2n	*P. ehr.*	From PE F_1 3 in culture	+	Tapered
PE F_1 6 2n	*P. ehr.*	From PE F_1 6 in culture	-	
PE F_2 3	*P. ehr.*	PE F_1 3 2n x PE F_1 6 2n	-	Tapered
PMYY 1 - 8	*P. mam.*	Yan Yean Resr., Vic.	+	
PMWB 1	*P. mam.*	Wurdee Boluc Resr., Vic.	+	
PMWB 1 2n	*P. mam.*	From PMWB 1 in culture	+	
PCGW 1	*P. cor.*	Goulburn Weir, Vic.	-	
PCGM 1	*P. cor.*	Glenmaggie Resr., Vic.	+	
PCGM 2	*P. cor.*	Glenmaggie Resr., Vic.	+	
PCWK 1	*P. cor.*	Wartook Resr., Vic.	+	
PCT 1	*P. cor.*	Tarago Resr., Vic.	+	
PCGW 1 2n	*P. cor.*	From PCGW 1 in culture	-	
PCWK 1 2n	*P. cor.*	From PCWK 1 in culture	+	

The mating types + and - are assigned arbitrarily.

* Clone will pair with plus clones but no conjugation has ever been observed.

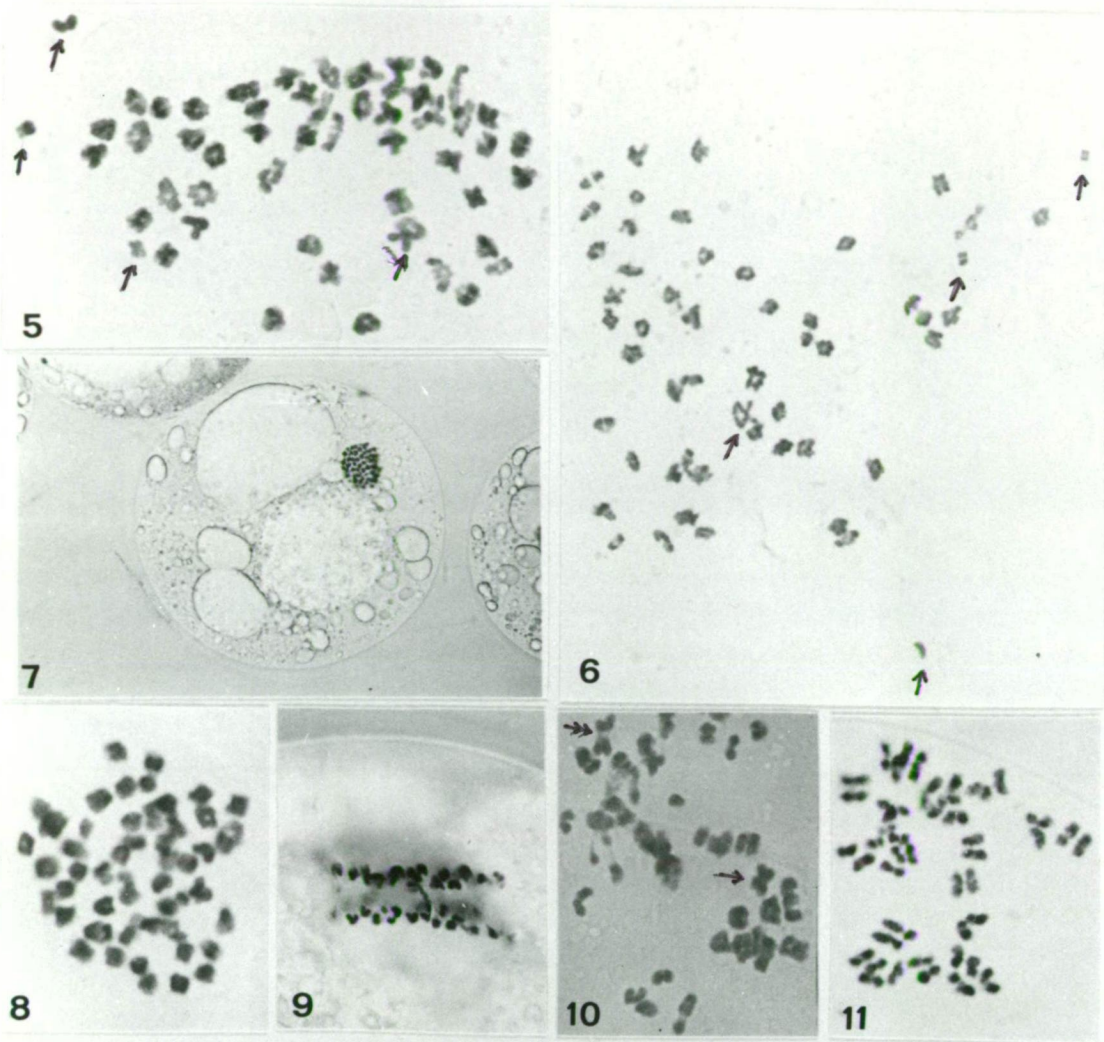


Fig. 5. Diakinesis. 53 bivalents, four of which appear to be morphologically distinct (arrows). x 1,250.
Fig. 6. Diakinesis. 53 bivalents. Same four morphologically distinct bivalents (arrows). x 900.
Fig. 7. Vesicle with single plastid, oil globules and a M I plate. x 320.
Fig. 8. M I, polar view. x 1,500.
Fig. 9. Synchronous A I. x 790.
Fig. 10. Asynchronous A I. Bivalents (single arrow), chromatid pairs end to end (double arrow) and individual chromatid pairs. x 1,500.
Fig. 11. Some M II chromosomes, most of which have a median to sub-median constriction. x 1,500.

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Sticky associations between bivalents are sometimes found at diplotene-diakinesis (Figs. 3, 4) but these do not persist to metaphase. Several preparations gave consistent counts of 53 bivalents (Figs. 3, 5, 6). In addition, four bivalents are morphologically distinct in some preparations - a large, somewhat omega-shaped bivalent, two small ones, and one that usually appears to be slightly bent forming a shallow v (Figs. 5, 6).

As diakinesis progresses the bivalents condense, shorten and move closer together until at metaphase I the thick, short, and darkly staining chromosomes form a flat, oval to circular plate (Fig. 7, 8).

Anaphase I is short-lived and therefore difficult to observe. Figure 9 shows a normal, synchronous anaphase, but there is evidence of asynchrony in some divisions (Fig. 10). Some bivalents are not fully terminalized (arrow) while others are on the verge of separation (double arrow). The majority of bivalents are still further advanced and have separated, with chromatids clearly visible.

At metaphase II (Fig. 11) the chromatids in a half bivalent are clearly visible, lying either parallel or slightly divergent from one another, most showing a median or sub-median constriction. A tiny strand sometimes bridges the pair. The chromosomes form an oval metaphase plate. In some preparations half bivalents may be seen to have separated completely into individual chromosomes while in the majority the chromatids are still associated. Consistent counts of 53 chromatid pairs were obtained from several second metaphases.

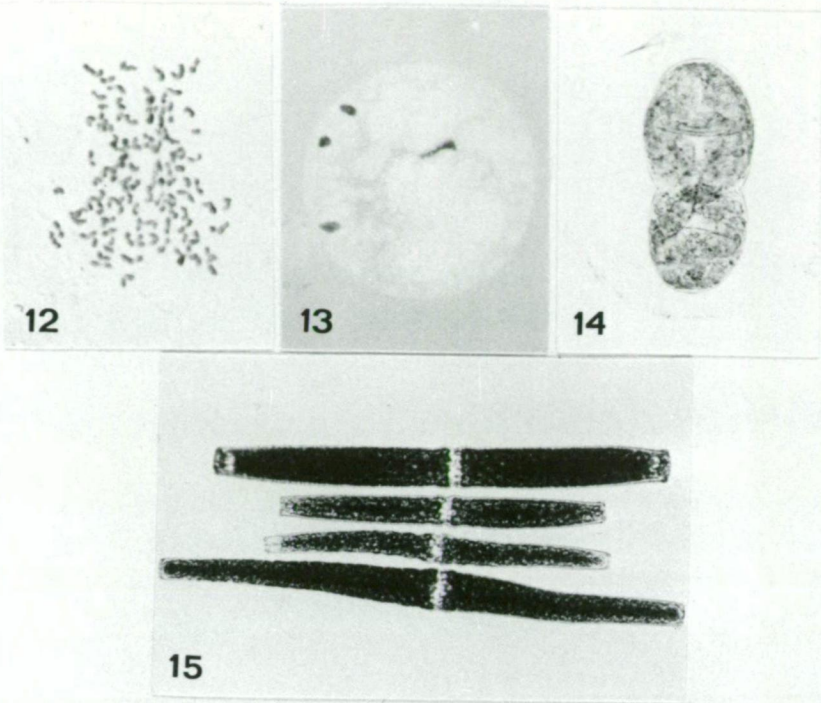


Fig. 12. Synchronous A II splayed out by squashing. x 1,000.

Fig. 13. Vesicle with 3 pycnotic nuclei at periphery and one centrally placed and fuzzy surviving nucleus. x 200.

Fig. 14. Single gone, with surviving nucleus in the isthmus. x 250.

Fig. 15. From top to bottom, diploid (PE F_1 6 2n), haploid (PEWL 2), tapered haploid (PE F_1 3) and tapered diploid (PE F_1 3 2n). x 135.

Figure 12 shows an early anaphase II where the separation of the chromatids appears to be synchronous, or nearly so. V-shaped chromatids are clearly visible. Anaphase II, like anaphase I, is short-lived. The chromosomes separate rapidly, the two anaphases in a single vesicle usually occurring at slightly different times.

At the end of anaphase II the chromosomes draw close together, losing their identity. The three aborting nuclei become dense and rounded and stain deeply. The chromosomes in the surviving nucleus become thread-like and stain lightly (Fig. 13).

Until now, the vesicle shows no external signs of the important events occurring internally. In the next few hours the protoplast condenses and draws away from the wall. A furrow develops and the one surviving nucleus occupies the isthmus of this single germination product (Fig. 14) while the small pycnotic nuclei may sometimes be seen towards the periphery of the semicells.

Seven F_1 clones, PE F_1 1 - 7, were isolated from several crosses of Tasmanian strains (Table 1). Clones 1, 3, 5 and 7 are plus in mating type, 4 and 6 are minus, and clone 2 shows a minus reaction. Clone 3 appears to be a mutant. All the cells in the clone are more attenuated towards the apices than normal cells (Fig. 15).

Meiosis in *P. ehrenbergii* x *P. mamillatum* crosses.

Meiosis in *P. ehrenbergii* x *P. mamillatum* zygospores is identical to meiosis in *P. ehrenbergii* x *P. ehrenbergii* zygospores. Counts of 53 bivalents/half-bivalents were consistently obtained from metaphases I/II, and the same four morphologically distinct bivalents were

TABLE 2

Dimensions (length x basal inflation x isthmus x apex um) of cultured strains of the *P. mamillatum* complex and wild type Wurdee Boluc Reservoir cells.

PEAL 1:	292-(334)-379 x 28-(30)-32 x 22-(24)-25 x 16-(18)-19
PE F_1 6 2n:	355-(418)-466 x 38-(42)-45 x 32-(36)-38 x 23-(26)-30
PE F_1 3:	306-(330)-358 x 32-(35)-39 x 26-(27.5)-32 x 16-(17)-19
PE F_1 3 2n:	340-(412)-525 x 40-(43)-46 x 30-(33)-36 x 19-(20)-23
PE F_2 3:	350-(423)-550 x 32-(38)-43 x 29-(32)-35 x 17-(19)-23
PCGW 1:	325-(402)-439 x 37-(40)-42 x 28-(31)-33 x 22-(24)-26
PCGW 1 2n:	480-(547)-648 x 42-(47)-53 x 34-(39)-43 x 25-(28)-32
Wurdee:	360-(398)-424 x 30-(32)-35 x 25-(26)-27 x 18-(19)-21

P. ehrenbergii

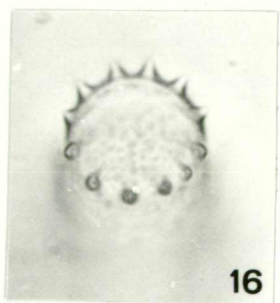


Fig. 16. End view of diploid cell (PE F_1 6 2n). x 750.

recognisable. The F_1 formed zygospores in mixed culture.

A presumed diploid was isolated from one of the F_1 cultures.

P. mamillatum, Wurdee Boluc Reservoir.

P. mamillatum has also been found in Wurdee Boluc Reservoir, Victoria (Map 1). Except for their smaller size these cells are identical to those from Yan Yean Reservoir (Table 2). The three clones isolated, PMWB 1 - 3, are all plus in mating type and have formed zygospores with PEWL 2. No germination or meiotic details are available.

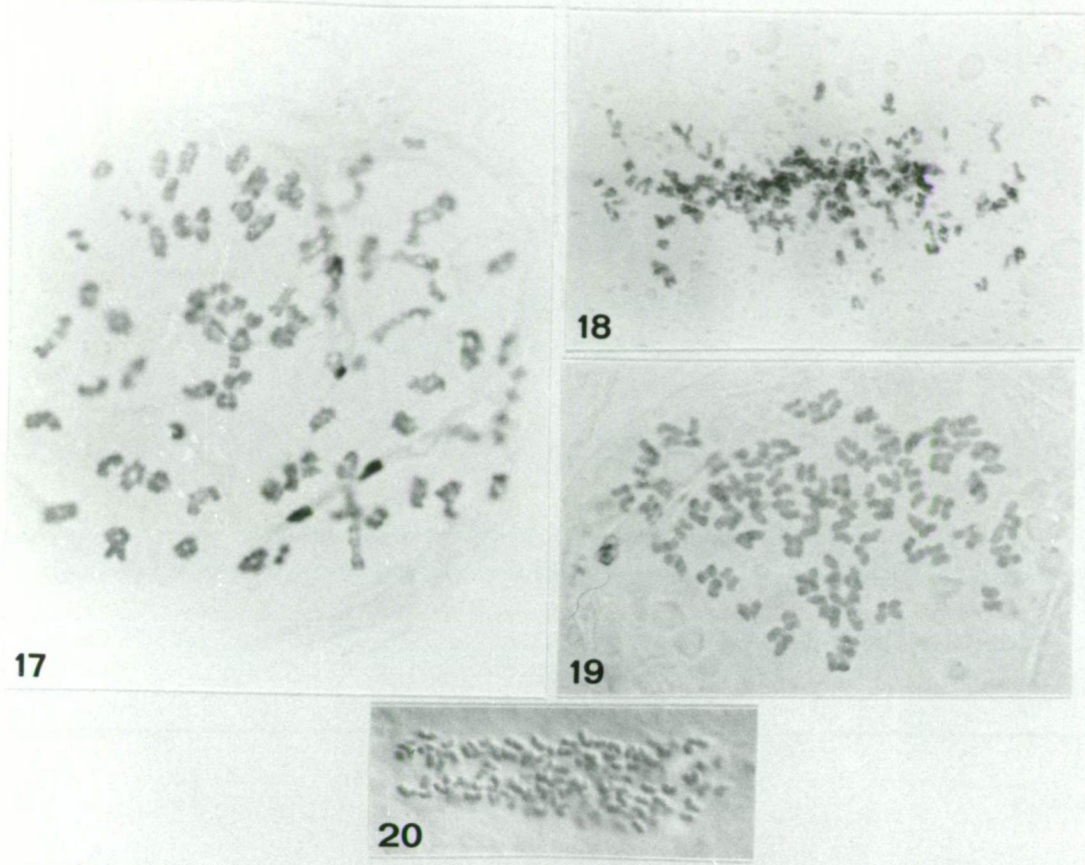
(ii). Polyploidy in *P. ehrenbergii* and *P. mamillatum*.

Diploid *P. ehrenbergii*.

Large cells arose spontaneously in three vegetative clones maintained in culture. From their large size (Fig. 15; Table 2) they are presumably diploids. They were isolated in clonal cultures as strains PE F_1 6 2n, PEAL 1 2n and PE F_1 3 2n (Table 1).

For unknown reasons, in the parent clone of strain PE F_1 6 2n, normal-sized cells died out, leaving a vigorous population of the large cells. In addition to larger size, they differ in having 11 - 15 tubercles (Fig. 16) instead of the 7 - 10 of the parent clone. No reversions to the normal cell type were observed. Clone PE F_1 3 2n retains the tapered morphology of its parent culture (Fig. 15).

Each of the three strains will form zygospores with either haploid or diploid strains of opposite mating type. On the whole, diploid x diploid crosses are more successful than diploid x haploid.



Figs. 17 - 20. Triploid meiosis in *P. ehrenbergii*.

Fig. 17. M I showing mainly bivalents and few uni- and trivalents. Omega-shaped bivalent at bottom left. x 1,300.

Fig. 18. Asynchronous A I. x 790.

Fig. 19. M II. x 1,400.

Fig. 20. Synchronous A II. x 1,000.

SO1/8/LL

In the latter, the haploid conjugants often produce papillae precociously, then lyse.

Germination of triploid zygospores of *P. ehrenbergii*.

Triploid zygospores resulting from the diploid x haploid crosses (PEAL 1 2n x PEWL 2; PE F_1 6 2n x PEAL 1/PEWL 1) germinate normally. The vesicles are released at about diakinesis. Chromosome association at this stage, though difficult to interpret because of interchromosomal stickiness (Fig. 17), apparently consists mainly of 65 - 70 bivalents, 8 - 12 univalents and a few presumptive trivalents.

During metaphase I the chromosomes line up. A few of the dissociated or unassociated chromosomes head for the poles, followed by more and more half bivalents (Fig. 18) as anaphase I progresses.

Figure 19 shows a metaphase II. Even though the chromatids in some of the half bivalents are slightly separated from one another, no individual chromatids are scattered about. Thus the chromatids of the metaphase I univalents do not separate until anaphase II.

Estimates of several metaphase II plates (e.g. Fig. 19) give counts of approximately 79, proving that the large cells are indeed diploids.

In the only anaphase II observed, separation of the chromatids appeared to be synchronous (Fig. 20). There was no stickiness and the chromatids were easily seen as individual rods. Approximately 150 - 160 chromosomes were counted.

Three of the resulting nuclei abort and a single product is formed. Triploid zygospores are only slightly less viable than

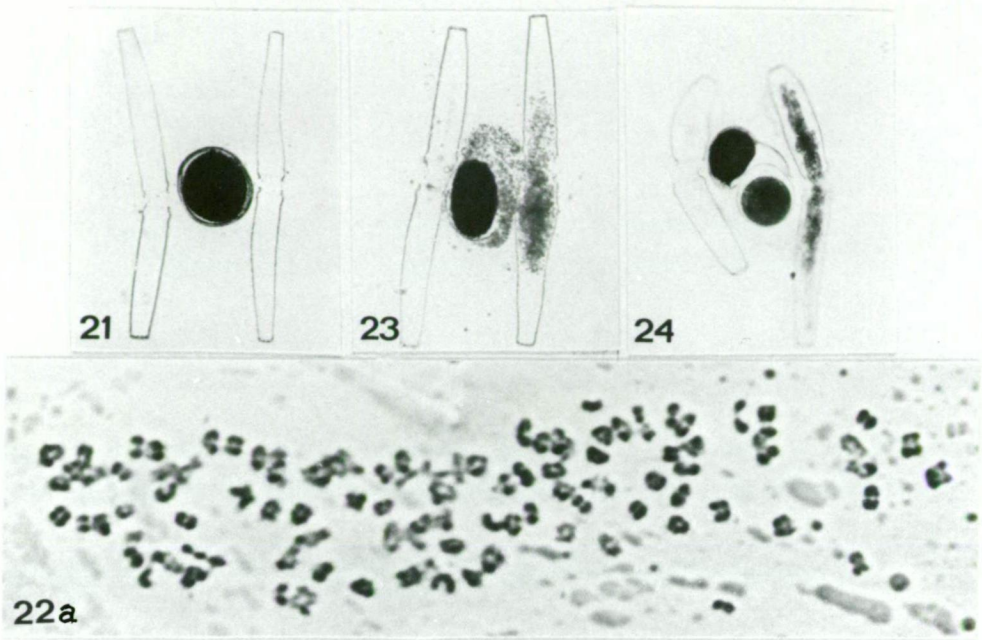


Fig. 21. Tetraploid zygospore (PE F_1 6 2n x PE F_1 3 2n). Tapered cell on right. x 100.

Fig. 22a. Tetraploid meiosis (PE F_1 6 2n x PE F_1 3 2n). M I with about 106 bivalents. x 1,170.

Fig. 23. PE F_1 6 2n x PE F_1 3 2n. "Parthenospore". Some lysed gamete material remains in the tapered cell. x 100.

Fig. 24. PE F_1 6 2n x PEAL 1 2n. "Parthenospore" germination. x 100.

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diploid ones. The F_1 is intermediate in size between the haploid and the diploid.

Germination of tetraploid zygospores of *P. ehrenbergii*.

Except for minor differences, zygospore germination and meiosis in tetraploid zygospores (Fig. 21) is identical to that in diploid zygospores. In one metaphase I (Fig. 22a, b) from a PE F_1 6 2n x PE F_1 3 2n, chromosomal associations consist of 106 presumed bivalents - an exact doubling of the diploid number of chromosomes. Thus all three giant cell clones are indeed diploids.

As in diploid and triploid zygospores a single product is formed. Like triploid zygospores, the tetraploid zygospores are only slightly less viable than diploid ones. Most of the mortalities occur during the early gonal divisions. Cells of the F_1, which maintain the large size, are interfertile.

"Parthenospores".

Crosses between PEAL 1 2n or PE F_1 3 2n with PE F_1 6 2n often produce some "parthenospores" as a result of lysis of one of a pair of gametes. When the cross involves the tapered PE F_1 3 2n it is possible to tell that most of the "parthenospores" are formed by PE F_1 6 2n gametes because of lysed material left in the tapered cells (Fig. 23). From one such cross fifty "parthenospores" were picked out and tested for germination - without any success. Germination of a few of these "parthenospores" from further crosses were observed, but the released vesicles failed to develop (Fig. 24). The "parthenospores" also have opercula (Fig. 24).

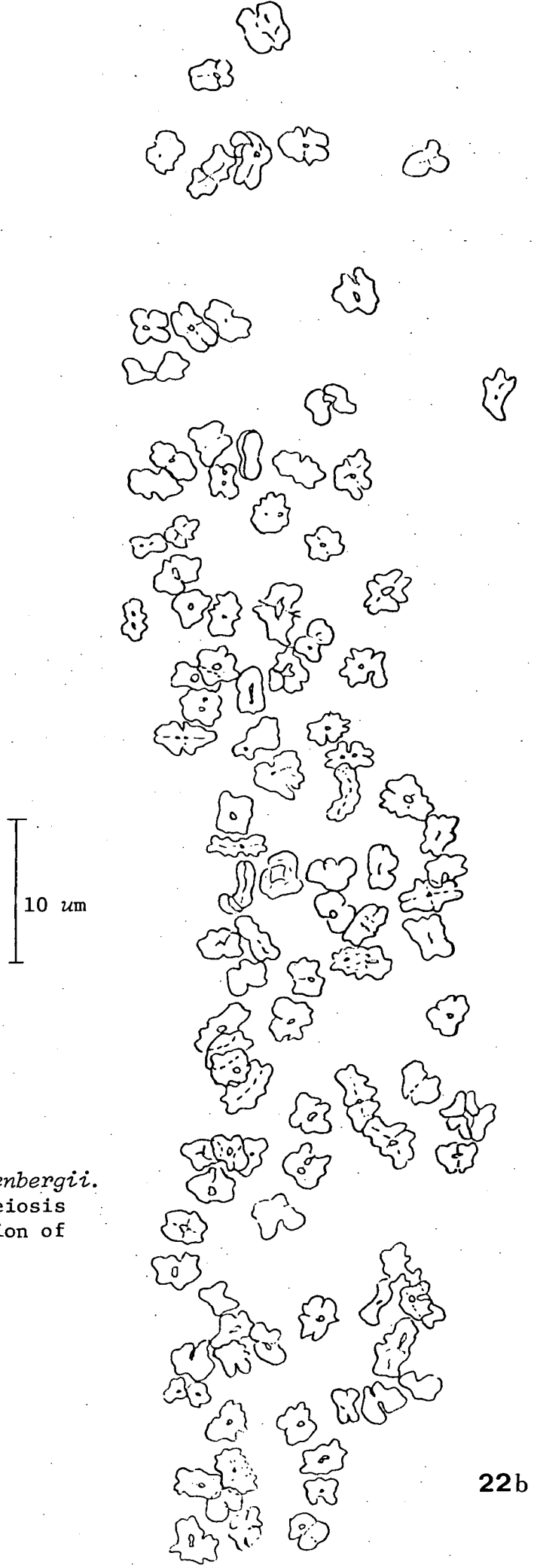

Fig. 22b. *P. ehrenbergii*. Tetraploid meiosis (interpretation of Fig. 22a).

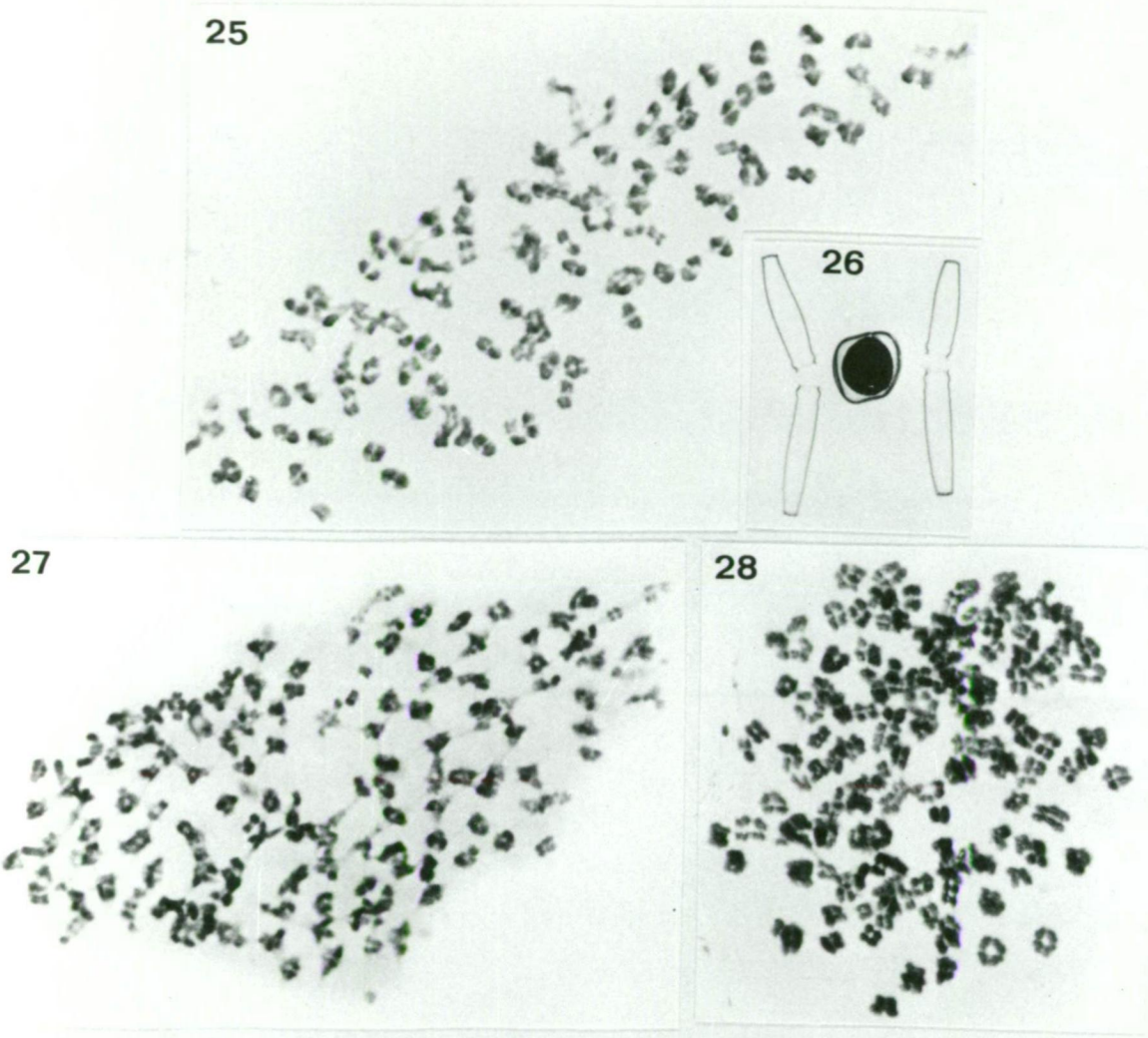

Fig. 25. Diploid *P. ehrenbergii* (PE F_1 6 2n) x diploid *P. mamillatum* (PMWB 1 2n) meiosis. M I ca. 106 bivalents. x 1,200.
Figs. 26 - 28. *P. coronatum* (PCGW 1) x diploid *P. ehrenbergii* (PEAL 1 2n).
Fig. 26. Zygospore. x 75.
Fig. 27. Diakinesis showing considerable stickiness. x 1,000.
Fig. 28. M I. x 1,300.

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Diploid *P. mamillatum.*

Clone PMWB 1 2n also originated from large cells arising spontaneously in PMWB 1. PMWB 1 2n has been crossed successfully with PE F_1 6 2n. About 106 bivalents were counted in one metaphase I preparation (Fig. 25). Cells of the F_1 maintain the large size of the parents.

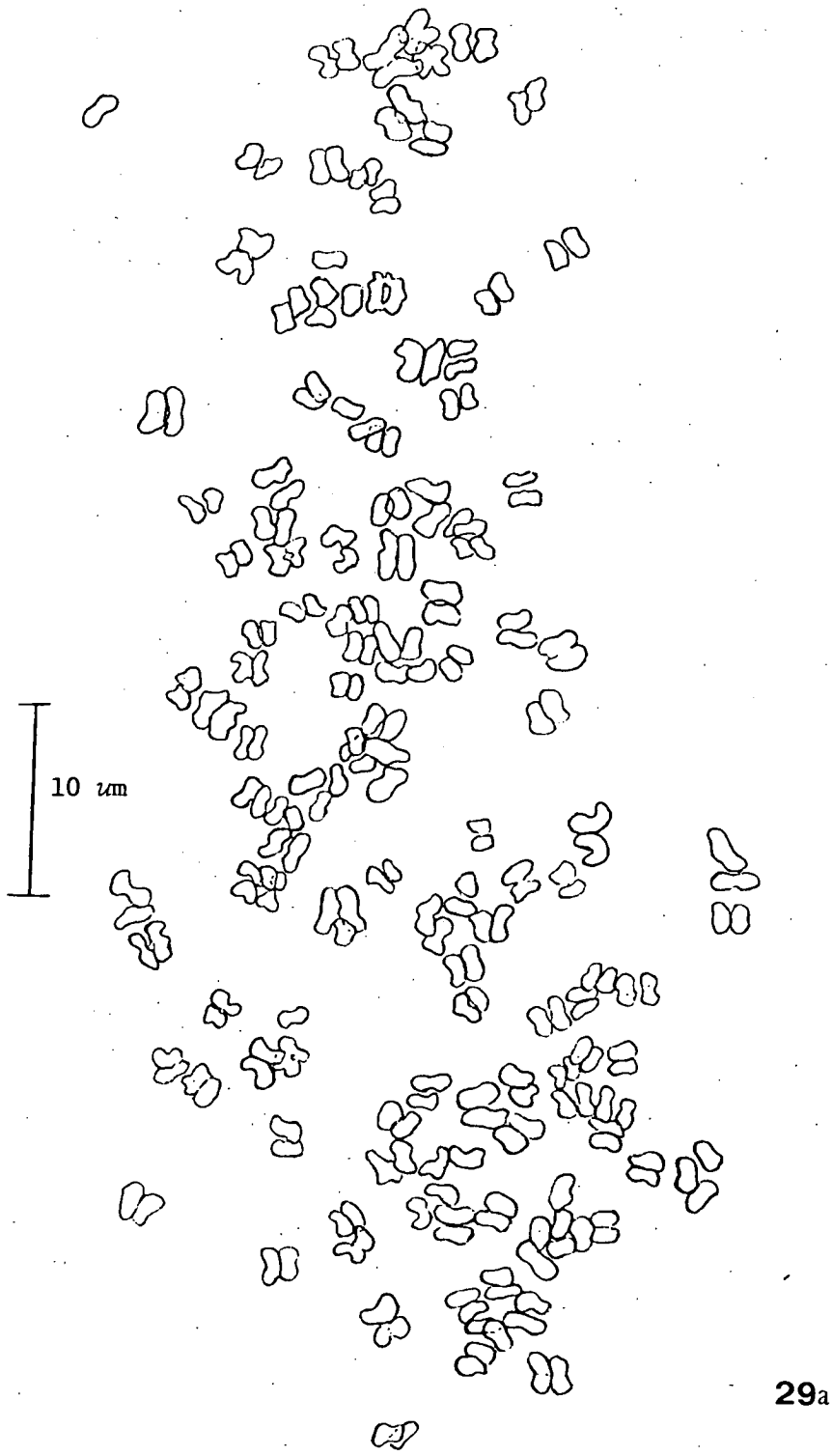
Miscellaneous diploids.

Recently, clones of presumed diploids were isolated from large cells in cultures of PELS 1, PEWL 2 and PE F_1 2.

(iii). *Pleurotaenium coronatum.*

Early evidence suggested that the Goulburn Weir clone, PCGW 1, identifiable as *P. coronatum* (Ling & Tyler, 1974), was a naturally occurring diploid strain of *P. ehrenbergii.* In culture it is identical (except for the slight difference in tubercle number) to a diploid clone, PE F_1 6 2n, of *P. ehrenbergii*, both morphologically and in sexual activity. Zygospores of PCGW 1 x PEAL 1 2n (Fig. 26) behave like tetraploid zygospores of *P. ehrenbergii.* They germinate at diakinesis and chromosome associations at diakinesis and metaphase consist mostly of bivalents. Stickiness is variable, and diminishes towards metaphase I (Figs. 27, 28).

Chromosome counts from six metaphase I vesicles indicate that *P. coronatum* is more complex than a simple diploid of *P. ehrenbergii.* Each vesicle contained 100 plus bivalents and a few uni-, tri-, and quadrivalents, totalling an equivalent of 122 - 130 bivalents. It was difficult to decide whether a small chromosome was a small bivalent, or a univalent, or whether a large chromosome was a large bivalent,

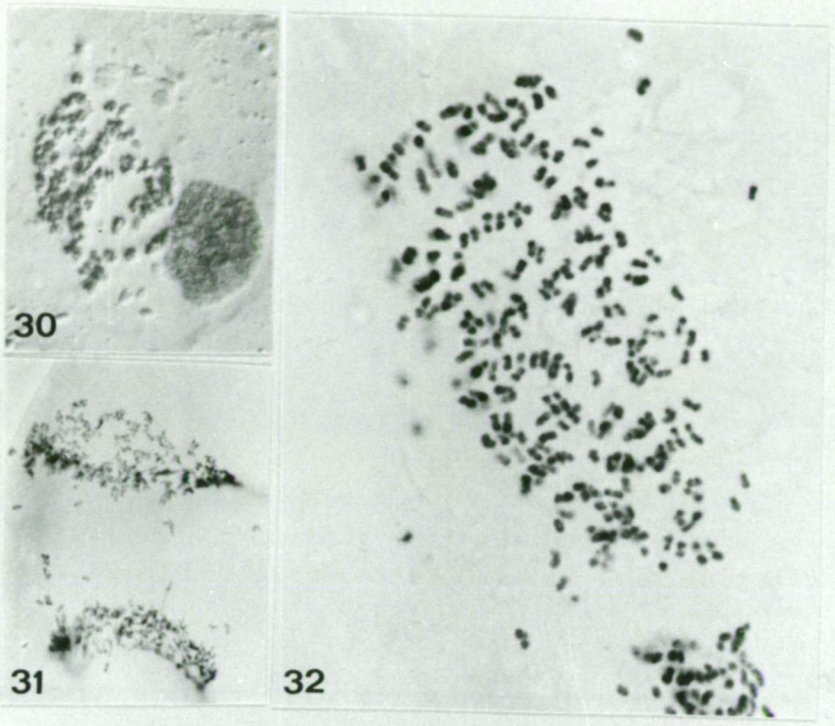


Figs. 29a, 29b. Interpretation of two metaphase II plates from a PCGW 1 x PEAL 1 2n vesicle.

Fig. 29a. First metaphase II plate.

29b

Fig. 29b. Second metaphase II plate.



Figs. 30 - 32. Meiosis in *P. coronatum* (PCGW 1) x diploid *P. ehrenbergii* (PEAL 1 2n).

Fig. 30. Non-fusion meiosis. M I chromosomes in close proximity to an aborted nucleus. x 750.

Fig. 31. A I with stickiness and laggards. x 600.

Fig. 32. Early A II. Asynchronous separation of chromatids. x 1,300.

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trivalent or quadrivalent. This difficulty was further compounded by stickiness (Fig. 27). In any case, the counts indicate an excessive number of bivalents, about 20 more than would be expected in a normal tetraploid zygospore. Accurate counts of 125 and 126 pairs of chromosomes respectively were later obtained from both metaphase II groups of a single vesicle (Figs. 29a, 29b).

It is evident that PCGW 1 has a larger chromosome complement than would be expected if it were merely a diploid *ehrenbergii*. PEAL 1 2n has been shown to have 106 chromosomes, and since PCGW 1 x PEAL 1 2n crosses give 125 - 126 pairs, **n** = 145 is the most probable number for PCGW 1, an unusual figure as it does not fit into any discrete *P. ehrenbergii* ploidy level.

In one of the preparations a most unusual nuclear configuration was observed - a group of metaphase I chromosomes in close proximity to what looks like an interphase nucleus (Fig. 30). Apparently nuclear fusion had not occurred, one nucleus aborting and the other proceeding into meiosis alone. It is not clear to which cell (*P. ehrenbergiii* 2n or *P. coronatum*) the surviving nucleus belonged because the chromosomes were clumped and could not be counted, but, of the chromosomes that were distinct, bivalents predominated. It would be most interesting to know if this type of meiosis were successful.

Figure 31 shows a late anaphase I. It appears to be asynchronous, with several half bivalents lagging behind. Stickiness is considerable. In an early anaphase II, separation of the chromatids was asynchronous (Fig. 32). A median constriction was seen clearly

in most of the chromatids.

At telophase the chromosomes move close together (Fig. 33). Three of the meiotic products abort leaving one surviving nucleus and hence one germination product. The F_1s, comparable to their parents in size, were grown on as a single mixed culture. Viable zygospores formed two months later. No significant differences were found between the initial meiosis and meioses in germinating zygospores formed by the F_1.

As stated previously (Ling & Tyler, 1974) in crosses between *P. coronatum* (PCGW 1) and haploid *P. ehrenbergii* (PE) each PCGW 1 cell attracted from 3 - 10 prospective conjugants. However, in crosses where the PCGW 1 cells are more than twice as many as PE, one, sometimes two PCGW 1 pair with one PE cell. Nevertheless, where the cell numbers are about equal, several PE cells still cluster around a single PCGW 1 cell.

Zygospores of PCGW 1 by haploid clones of *P. ehrenbergii* have been germinated successfully. Estimates of 95 - 100 half bivalents each were made of both metaphase II from one vesicle. The F_1s are intermediate in size between the parents and are interfertile.

P. coronatum, Glenmaggie Reservoir.

Clone PCGM 1, morphologically identical to PCGW 1, was isolated from Glenmaggie Reservoir about 200 km from Goulburn Weir, in a different catchment (Map 1). The clone will conjugate with PCGW 1 and compatible haploid or diploid clones of *P. ehrenbergii*.

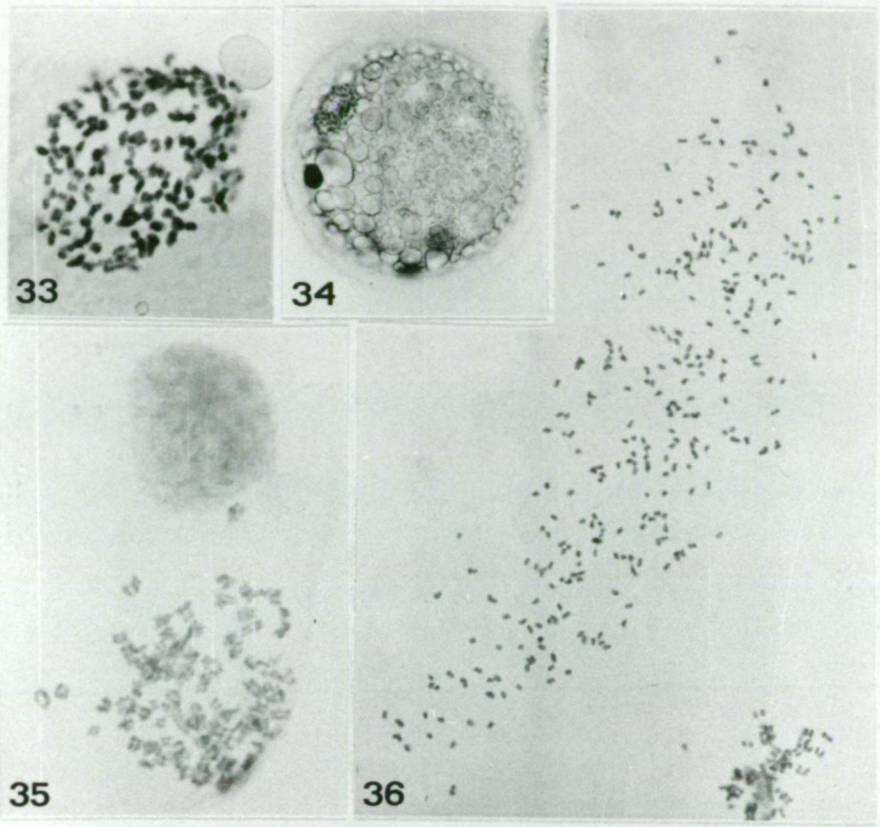

Fig. 33. PCGW 1 x PEAL 1 2n. Late A II. x 1,000.

Figs. 34 - 36. Meiosis in *P. coronatum* (PCGW 1 x PCGM 1).

Fig. 34. Vesicle with lightly stained surviving nucleus and three darkly stained aborted nuclei (two out of focus at 6 o'clock). x 250.

Fig. 35. Non-fusion meiosis. Chromosomes predominantly bivalents. x 860.

Fig. 36a. Early A II. x 780.

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Zygospores of Goulburn x Glenmaggie crosses were germinated successfully. The process of meiosis is similar to that in tetraploid zygospores of *P. ehrenbergii*. Bivalents dominate. Three of the nuclei abort becoming round, dense and dark staining. The surviving nucleus stains lightly (Fig. 34). The F_1s are interfertile.

The most interesting feature is again the observation of some non-fusion meiosis stages. Figure 35 shows the first division in a released vesicle. One nucleus has aborted while the other clearly shows bivalents.

From an early anaphase II (Figs. 36a, 36b) a count of 294 individual chromosomes was obtained, i.e. n = 147 for *P. coronatum*. This is only two more than the previous estimate of n = 145.

Other *P. coronatum* clones.

Three other clones PCGM 2, PCWK 1 and PCT 1 were isolated from Glenmaggie, Wartook and Tarago Reservoirs, Victoria respectively. All three clones are plus in mating type. PCGM 2 is probably identical to PCGM 1.

Diploid *P. coronatum*.

From PCGW 1 an enormous cell was isolated which multiplied to form a clone, PCGW 1 2n. Cells in the clone retain their enormous size (Table 2) and have 11 - (13) - 15 apical tubercles. This clone produces more zygospores with the large-celled clones PCGM 1, PEAL 1 2n and PE F_1 3 2n than with the small-celled haploids PELS 1, PEAL 1 and PMYY 1. Indeed, with the two latter clones very few zygospores are formed. In the crosses each PCGW 1 2n cell usually attracts a large number of

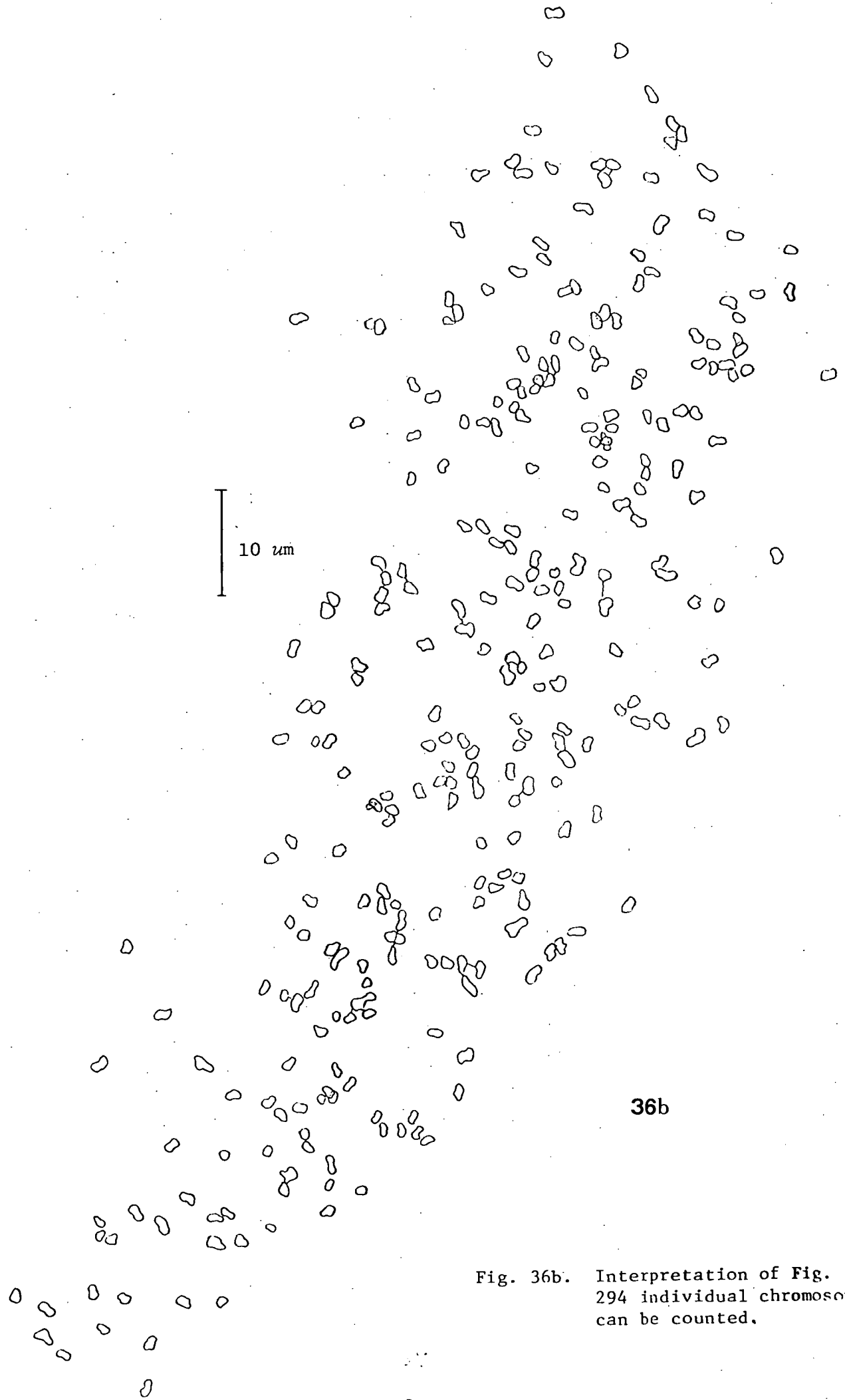
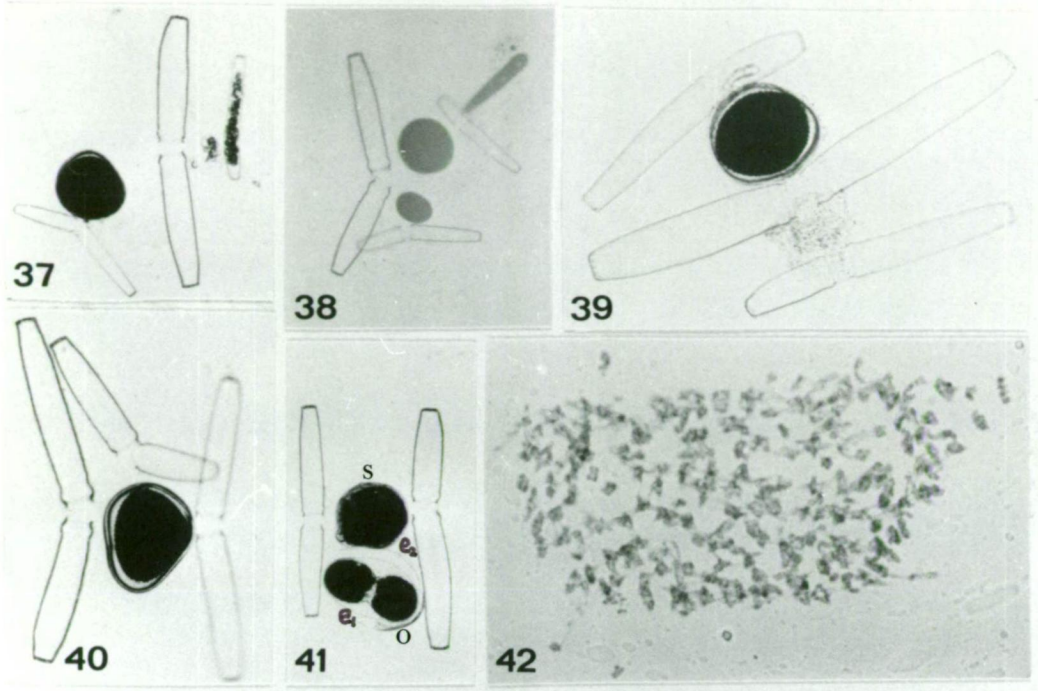


Fig. 36b. Interpretation of Fig. 36a. 294 individual chromosomes can be counted.



Figs. 37 - 38. Haploid *P. ehrenbergii* (PELS 1) x diploid *P. coronatum* (PCGW 1 2n).

Fig. 37. One n *P. ehrenbergii* cell has formed a zygospore with the 2n *P. coronatum*. The gamete from the other *ehrenbergii* cell has lysed. x 75.

Fig. 38. Zygospore between n *P. ehrenbergii* and 2n *P. coronatum*. The 2nd *ehrenbergii* cell formed a parthenospore, the 3rd took part only in pairing. x 60.

Figs. 39 - 42. Diploid *P. ehrenbergii* (PEAL 1 2n) x diploid *P. coronatum* (PCGW 1 2n).

Fig. 39. Only one of the two 2n *P. ehrenbergii* cells is successful in forming a zygospore with the 2n *P. coronatum* cell. x 125.

Fig. 40. Zygospore from two 2n *P. ehrenbergii* cells with a single 2n *P. coronatum* cell. x 100.

Fig. 41. Single gone from 2n *P. ehrenbergii* x 2n *P. coronatum* zygospore. s = spore coats; e_1, e_2 = endospores; 0 = operculum. x 75.

Fig. 42. 2n *P. ehrenbergii* x 2n *P. coronatum* meiosis. Diakinesis. x 840.

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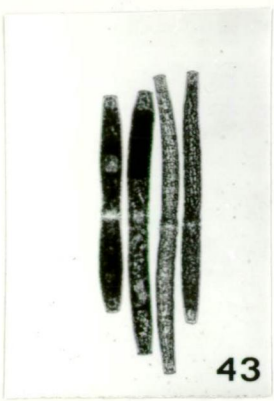
prospective conjugants, none, only one or very rarely two of which forms a zygospore with it. The others produce conjugation papillae then lyse, or very rarely, form a parthenospore (Figs. 37 - 40). The result is a mixture of lysed PEAL 1 2n cells (or PELS 1, etc.), some zygospores, and the PCGW 1 2n cells still alive and unconjugated. As the cell numbers of PEAL 1 2n or PELS 1 drop to well below those of PCGW 1 2n, a dramatic reversal occurs. Each PEAL 1 2n or PELS 1 cell is then surrounded by several PCGW 1 2n cells. In crosses where the initial concentration of PCGW 1 2n cells was more than twice as many as PELS 1 cells, each PELS 1 cell was surrounded by from 1 - 4 PCGW 1 2n cells.

Zygospores from PCGW 1 2n by PCGM 1, PEAL 1 2n and PELS 1 were germinated successfully (Fig. 41). Rough estimates of chromosome numbers in the germination vesicles (Fig. 42) are consistent with the assumption that the clone PCGW 1 2n is a diploid strain of PCGW 1.

Recently, PCWK 1 2n was isolated from PCWK 1. The first few cells were sub-cultured in soil-water media 46 and 14R in watch-glasses. Cells in 46 were slender whereas those in 14R were stout (Fig. 43). When subsequently transferred to larger volumes of media in petri-dishes, or if a few cells from 46 were transferred into 14R and vice versa, the new semicells assume an intermediate shape.

PCWK 1 2n is slightly larger than PCGW 1 2n and was crossed successfully with it (Fig. 44).

Table 3 summarises the results of strain lineages, crosses, etc.



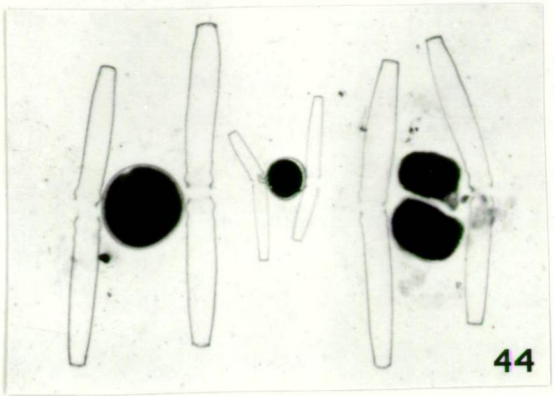


Fig. 43. Stout (two cells on left) and slender cells of diploid *P. coronatum* (PCWK 1 2n). x 60.

Fig. 44. Diploid *P. coronatum* (PCGW 1 2n x PCWK 1 2n) zygospore and "parthenospores". A *P. ehrenbergii* zygospore (centre) is included for comparison. x 75.

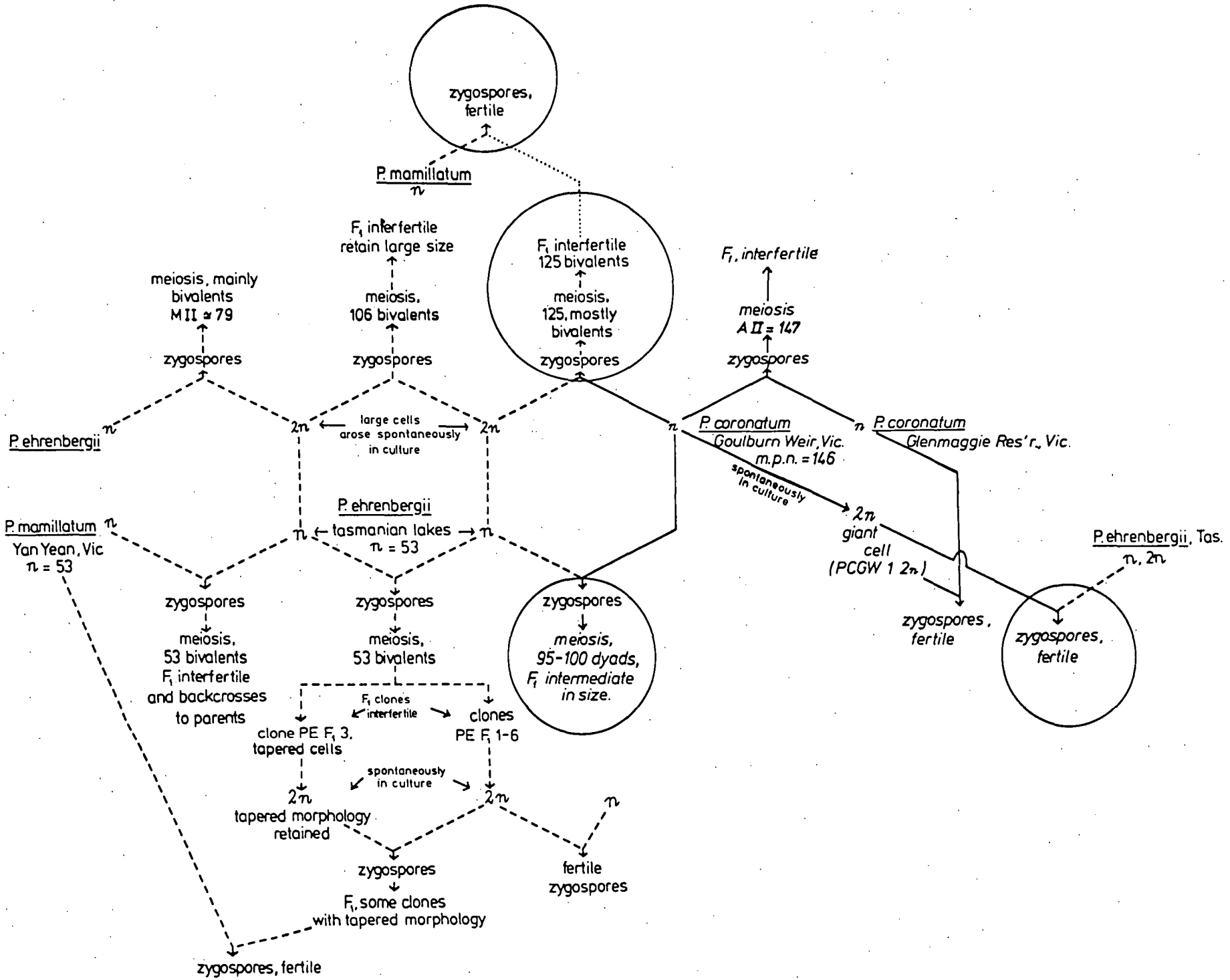


Table 3. Diagram showing compatibility, fertility and some chromosome details for the *P. mamillatum* complex.

(iv). Inheritance of the tapered character.

Cells of one of the F_1 clones, PE F_1 3, were significantly more tapered than normal cells (Fig. 15). Unlike a normal cultured cell which is fairly uniform in width till very near the apex where it narrows suddenly, a tapered cell has a slightly wider basal inflation and tapers all the way to a slightly narrower apex (Table 2).

Tapered cells are slow growing and often lyse in the process of division. The clone was mated successfully with PE F_1 4 and PEAL 1. Attempts at germinating zygospores from both crosses were unsuccessful. PE F_1 3 maintained its tapered morphology for about 18 months when it gradually began to revert to normal. The present culture is a mixture of normal, intermediate and tapered cells.

Clone PE F_1 3 2n was raised from a large cell which arose spontaneously in culture. The cells maintain the large size, with a more pronounced tapering (Fig. 15; Table 2).

Germination of PE F_1 3 2n x PE F_1 6 2n zygospores is mentioned on page 28 where it is shown that PE F_1 3 2n has more or less double the haploid number of chromosomes. It thus seems that the tapered character is not caused by extra chromosomes or a deficient complement.

Cells of the F_1 (PE F_1 3 2n x PE F_1 6 2n) are all large. The tapered character is transmitted to the F_1 and usually manifests itself in the first gonal division. Out of seven F_1 clones, four are tapered, one appears to be an intermediate and two are normal. One of the tapered clones, PE F_2 3, is slightly more slender than its tapered parent (Table 2). There are considerable inter- and intra-clonal differences in size and slenderness which are intensified or attenuated

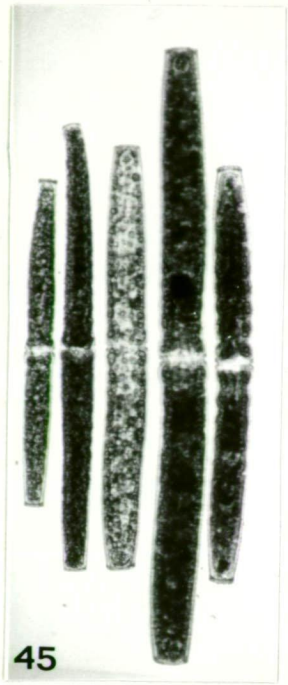


Fig. 45. Strains used in determining selective mating behaviour. From left to right; Class A - haploid *P. ehrenbergii* (PELS 1), Class B(i) - tapered diploid *P. ehrenbergii* (PE F_1 3 2n), Class B(ii) - normal diploid *P. ehrenbergii* (PE F_1 6 2n), Class C - diploid *P. coronatum* (PCGW 1 2n) and Class B(iii) - haploid *P. coronatum* (PCGM 1). x 130.

by differences in soil-water media. The two normal F_1 clones are more stable. The tapered character is also found in the F_1 of PE F_1 3 2n x PCGW 1.

PE F_1 3 2n has, since lost much of its length and slenderness. Though the clone is still tapered, many of the cells are difficult to distinguish from normal.

(v). Non-conjugating *P. ehrenbergii* F_1 clone.

As mentioned previously (page 22), one of the *P. ehrenbergii* F_1 clones, PE F_1 2, shows a minus type reaction. Whenever it is mixed with plus clones, including one of its parents, "text-book" type pairing takes place, but not once did a single conjugation or even papillae initiation occur in spite of repeated crossings. The clone shows no reaction with minus clones.

PE F_1 2 was then used in multiple crosses (Table 4) in an attempt to find out more about its behaviour. In each cross (3, 8) it again participated only in pairing. In cross 23 it and another minus clone formed triple associations with a plus clone. It remained inactive even though the other two cells were actively conjugating.

(vi). Selective mating.

Morphological differences among the various clones (Fig. 45) serve as convenient markers in multiple crosses, where in each cross three or more clones are mixed together. The clones can be split into three classes on size differences. Class A contains the small haploid *P. ehrenbergii* and *P. mamillatum*. Class B contains the medium-sized diploid *P. ehrenbergii* and haploid *P. coronatum*. This class can be

divided further into three sub-classes, encompassing (i) the tapered diploid, (ii) the normal diploid and (iii) the haploid *P. coronatum*. To distinguish between (ii) and (iii) the tedious task of counting the number of apical tubercles is resorted to. An additional aid is to produce long and slender (ii) in contrast to stout and more undulate (iii) by growing them in different media. Class C contains the large diploid *P. coronatum*.

The crosses and results are listed in Table 4. In crosses 5, 13 and 15 two compatible clones were mixed first. Only after the cells in the mixture have paired was the third clone (clone in brackets) added.

Though every plus clone has not been tested against every minus clone, opposite mating clones in each multiple cross have been mated successfully. PCWK 1 2n is the only exception because only a small number of cells was available.

Out of 23 crosses at least 20 indicate some form of selective pairing. In crosses 1 - 4, 6 and 7 *P. ehrenbergii* clones preferred other clones of the same ploidy level, singling out mates of the same size (clearly indicated by crosses 9, 16, 19) or closest to them in size (crosses 20, 21). This preference is strong enough to disrupt previously paired cells (crosses 5, 13, 15). However, this preference for similar size mates can be over-ruled by the attractiveness of particular clones (crosses 8, 23). Some clones appear to be more attractive than others. PEWL 2 perhaps is an attractive clone, PEF_1 2 certainly is (cross 3). Among clones of the same size, PEF_1 2 is more attractive than PEF_1 6 2n (cross 8) while PEAL 1 2n is more attractive than PEF_1 3 2n (cross 10).

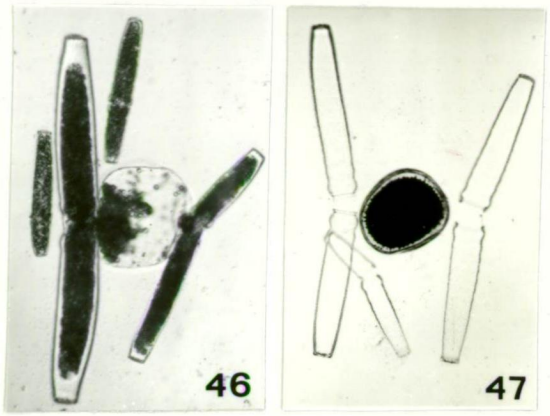


Fig. 46. Multiple pairing in a mixture of PEAL 1, PEAL 1 2n and PCGW 1 2n. Conjugation has taken place between PEAL 1 2n and PCGW 1 2n. x 100.

Fig. 47. Zygospore formed by conjugation between PEAL 1, PEAL 1 2n and PCGW 1 2n. x 100.

Crosses 11, 12 and 13 show that *P. coronatum* prefer *P. coronatum* to *P. ehrenbergii* clones whereas crosses 14, 15 and 16 show that *P. ehrenbergii* prefer *P. ehrenbergii* to *P. coronatum* clones. Cross 21 effectively supports both these findings.

No selective pairing was observed in either cross 17 or 18. Apparently, from the zygospore results, conjugations between some clones are more successful than between others.

Cross 22 introduces another factor governing selective mating, the activeness of clones. PEAL 1 2n is more successful than PCWK 1 2n in pairing with PCGW 1 2n presumably because it is more active.

Fig. 46 shows multiple pairing in a mixture of PEAL 1, PEAL 1 2n and PCGW 1 2n. Conjugation has taken place between PEAL 1 2n and PCGW 1 2n. Fig. 47 shows a rare event, a zygospore formed by conjugation between a haploid *P. ehrenbergii*, a diploid *P. ehrenbergii* and a diploid *P. coronatum*.

The bulk of the results on the *P. mamillatum* complex has been published: LING, H.U. & TYLER, P.A., 1976. Meiosis, polyploidy and taxonomy of the *Pleurotaenium mamillatum* complex (Desmidiaceae). Br. Phycol. J., 11 : 315-330.

Table 4. Multiple crosses and results.

	+		-	Results
1.	PELS 1	x	PEWL 2 PEF_1 6 2n	PELS 1 x PEWL 2 P & Z +++ PELS 1 x PEF_1 6 2n P & Z an odd one or two.
2.	PELS 1 PEAL 1 2n	x	PEWL 2	PELS 1 x PEWL 2 P & Z +++ PEAL 1 2n x PEWL 2 P & Z few, Z formed mostly when PELS 1 in short supply, excess PEWL 2.
3.	PELS 1	x	PEF_1 2 PEF_1 6 2n	PELS 1 x PEF_1 2 P +++ No Z PELS 1 x PEF_1 6 2n P & Z few.
4.	PELS 1	x	PEWL 2 PCGW 1	PELS 1 x PEWL 2 P & Z +++ PELS 1 x PCGW 1 P & Z an odd one or two.
5.	PELS 1	x	PCGW 1 (PEWL 2)	PELS 1 x PCGW 1 P ++ Few Z PELS 1 x PEWL 2 P & Z +++.
6.	PELS 1 PEAL 1 2n	x	PEWL 2 PEF_1 6 2n	a. PELS 1 x PEWL 2 P & Z +++ b. n x 2n P & Z some PEAL 1 2n x PEF_1 6 2n P & Z ++ Z formed mostly a few days after a and b.
7.	PEAL 1 PEAL 1 2n	x	PEF_1 6 2n	a. PEAL 1 x PEF_1 6 2n b. PEAL 1 2n x PEF_1 6 2n Z ratio a : b = 1 : 4.
8.	PEF_1 3 2n	x	PEF_1 2 PEF_1 6 2n	PEF_1 3 2n x PEF_1 2 P +++ No Z PEF_1 3 2n x PEF_1 6 2n P ++ Some Z.
9.	PEAL 1 2n	x	PEF_2 3 PEF_1 6 2n	PEAL 1 2n x PEF_2 3 P & Z ++ PEAL 1 2n x PEF_1 6 2n P & Z some. Z mostly when PEF_2 3 in short supply, excess PEAL 1 2n.
10.	PEAL 1 2n PEF_1 3 2n	x	PEF_2 3	PEAL 1 2n x PEF_2 3 P & Z ++ PEF_1 3 2n x PEF_2 3 P & Z an odd one or two.
11.	PCGM 1	x	PCGW 1 PEF_1 6 2n	PCGM 1 x PCGW 1 20 zygospores PCGM 1 x PEF_1 6 2n 0 zygospores out of a total of 20 zygospores looked at.

P = pairing, C = conjugation, Z = zygospore

Table 4. Continued.

12.	PCGM 1 x PEF$_2$ 3 PCGW 1		PCGM 1 x PEF$_2$ 3 P & Z none in one cross, an odd few in two other crosses PCGM 1 x PCGW 1 P & Z +++.
13.	PCGM 1 x PEF$_2$ 3 (PCGW 1)		PCGM 1 x PEF$_2$ 3 P & Z few in each of three crosses PCGM 1 x PCGW 1 P & Z +++.
14.	PELS 1 x PEWL 2 PCGW 1 2n		PELS 1 x PEWL 2 P & Z +++ PELS 1 x PCGW 1 2n P + No Z.
15.	PELS 1 x PCGW 1 2n (PEWL 2)		PELS 1 x PCGW 1 2n P +++, C +, No Z PELS 1 x PEWL 2 P & Z +++.
16.	PMYY 3 x PEWL 2 PCGW 1 2n		PMYY 3 x PEWL 2 P & Z +++ PMYY 3 x PCGW 1 2n P + No C.
17.	PELS 1 x PCGW 1 2n PCWK 1		PELS 1 x PCGW 1 2n P ++, C +, Few Z PCWK 1 x PCGW 1 2n P & Z ++.
18.	PEAL 1 x PCGW 1 2n PEAL 1 2n		PEAL 1 x PCGW 1 2n P ++, C +, Few Z PEAL 1 2n x PCGW 1 2n P & Z ++.
19.	PCGM 1 x PCGW 1 PCGW 1 2n		PCGM 1 x PCGW 1 P & Z ++ PCGM 1 x PCGW 1 2n P & Z some, Z mostly when PCGW 1 in short supply, excess PCGM 1.
20.	PMYY 3 x PEF$_1$ 6 2n PEAL 1 2n PCGW 1 2n		PMYY 3 x PEF$_1$ 6 2n zygospores 13 PMYY 3 x PCGW 1 2n 1 PEAL 1 2n x PEF$_1$ 6 2n 72 PEAL 1 2n x PCGW 1 2n 14 out of a total of 100 zygospores looked at.
21.	PELS 1 x PEF$_2$ 3 PCGM 1 PCGW 1		PELS 1 x PEF$_2$ 3 P +++ Z 86 PELS 1 x PCGW 1 P + Z 14 PCGM 1 x PEF$_2$ 3 P few Z 6 PCGM 1 x PCGW 1 P +++ Z 125 out of a total of 231 zygospores looked at.
22.	PEAL 1 2n x PCGW 1 2n PCWK 1 2n		PEAL 1 2n x PCGW 1 2n P +++, C +++, Z + When PEAL 1 2n exhausted through precocious release of gametes and lysis then PCWK 1 2n x PCGW 1 2n P & Z ++.

Table 4. Continued.

23.	PEAL 1 2n x PEF_1 2 PEF_1 6 2n	Pairing initially PEF_1 2 x PEAL 1 2n, subsequently PEF_1 6 2n joins PEF_1 2 and PEAL 1 2n in triple association. Conjugation and zygospores between PEF_1 6 2n and PEAL 1 2n only.

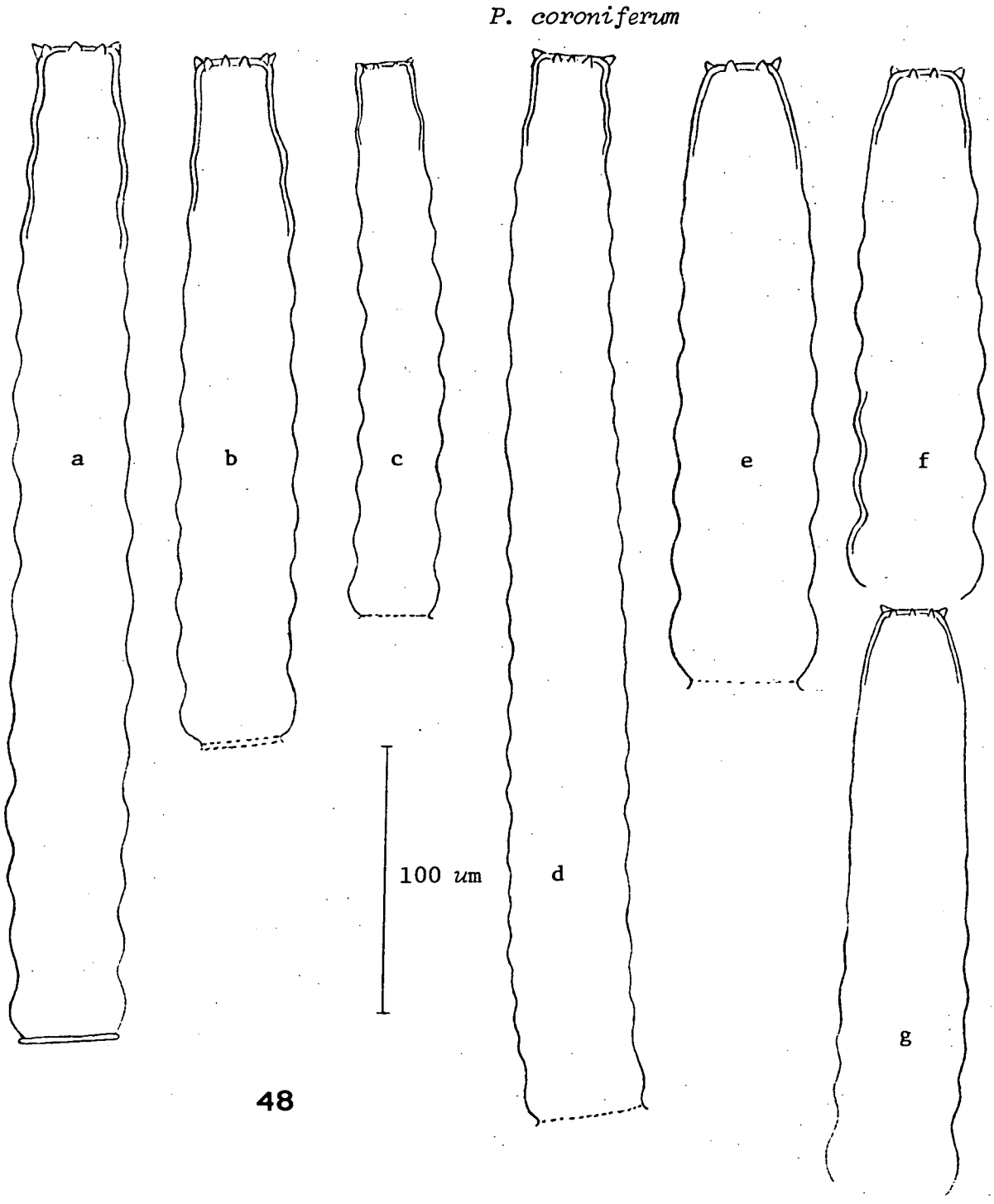


Fig. 48. *P. coroniferum*, a and b are semicells of a single dead cell from Tarago; c is from T 1 in culture; d is from the diploid clone T 1 2n; e and f are wild type Pine Lake; g is from a Pine Lake culture.

2. *Pleurotaenium coroniferum* (Borge) Krieg.

Taxonomy.

The culture, T 1, was obtained from Joan Powling as *P. coroniferum*. The original cell that started the culture was isolated from Tarago Reservoir, Victoria. In a recent Tarago sample a single dead cell (subsequently separated into semicells) was found (Figs. 48a, 48b, 49). Semicells 48a and 48b have 10 and 9 apical tubercles respectively (Fig. 50). From the dimensions (Table 5) it seems highly likely that the original cell of T 1 resembled this dead cell, probably came from the same population and had small semicells like 48b.

The plants agree with dimensions and general shape of *P. coroniferum* given by Krieger (1937, p. 422; Pl. 45, Fig. 9). However, Krieger described *P. coroniferum* semicells as having 6 - 8 undulations and 5 - 7 small, apical beads. From Krieger's figure it seems the 5 - 7 apical beads are actually those observed side on, not the total number on the apex. Hinode (1969) described a rather larger and more elongate Japanese *P. coroniferum* which had 14 - 15 minute apical tubercles.

T 1 semicells, though significantly smaller than wild cells (Fig. 48; Table 5) still have 9 - 11 undulations and 7 - (7.9) - 9 apical tubercles.

From three Victorian localities, viz a service basin in Nyah West, Wartook Reservoir and Pine lake, another *Pleurotaenium* was found (Fig. 48, e - g). The cells are stout. Each semicell has 7 - 9 undulations and is rather suddenly attenuated to an apex bearing 6 - (7.8) - 9

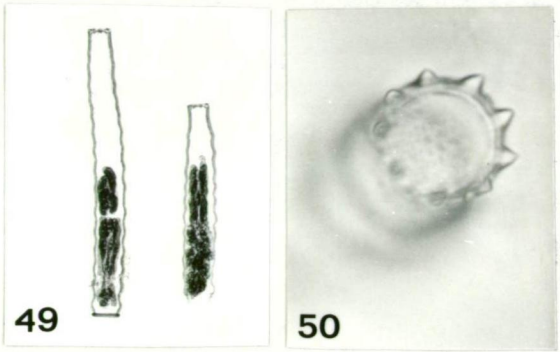


Fig. 49. Semicells of a single dead cell from Tarago Reservoir. x 125.

Fig. 50. End view of longer semicell in Fig. 49. x 750.

Table 5. Dimensions of *P. coroniferum* and its varieties (μm).

Source	length	basal inflation	isthmus	apex
P. coroniferum				
Krieger, 1937	350–483	32–39	–	20–25
Hinode, 1969	545–645	40–41	–	–
Tarago, dead cell	512	33	24	21
Clone T 1 initial cell	425	–	25	20
Clone T 1	251–(306)–331	24–(25.5)–27	19–(20)–21	14–(16.5)–18
Clone T 1 2n	457–(529)–576	32–(35.5)–37	28–(30)–32	18–(21)–24
Pine lake, Vic.	319–(333)–376	40–(42)–46	27–(29)–32	20–(22)–27
Clone W 1	239–(287)–331	35–(39)–43	27–(29)–31	18–(19)–20
Clone N 2 2n	418–(439)–475	50–(53)–56	42–(43)–45	24–(26)–29
P. coroniferum var. *cuyabense*				
Krieger, 1937	307–489	39–40.5	–	25–26

tubercles. The plants agree with descriptions and figures of *P. coroniferum* var. *cuyabense* given by Krieger (1937, p. 422 & Pl. 45, Fig. 11) except for apical ornamentation. Krieger's figure has 4 small apical beads. For want of a better identification and from the results to follow, the plants will be lumped with the Tarago ones as *P. coroniferum*.

Clone W 1 was raised from one of two cells in the Wartook sample. Clones N 1 and N 2 are from Nyah. A few young clones were isolated from Pine Lake. Cultured cells of all the clones are more slender and less undulate than wild cells.

Conjugation.

All clones isolated are heterothallic. W 1, N 1 and N 2 are all of the same mating type and will cross with T 1. Attempts to cross *P. coroniferum* clones with clones from the *P. mamillatum* complex were unsuccessful.

T 1 x W 1. Pairing depends on the relative numbers of T 1 to W 1 cells. When T 1 > W 1 several T 1 cells congregate round each W 1 cell. When T 1 < W 1 each T 1 cell attracts from one to two W 1 cells. In either situation (more so in the former) many of the T 1 gametes lyse probably because of precociousness. Successful conjugation is normally in a one to one ratio.

The conjugation process and zygospore characteristics are the same as those described for *Pleurotaenium* species in general. The operculum is smooth.

T 1 x N 1 or N 2. Multiple conjugations are common in T 1 by

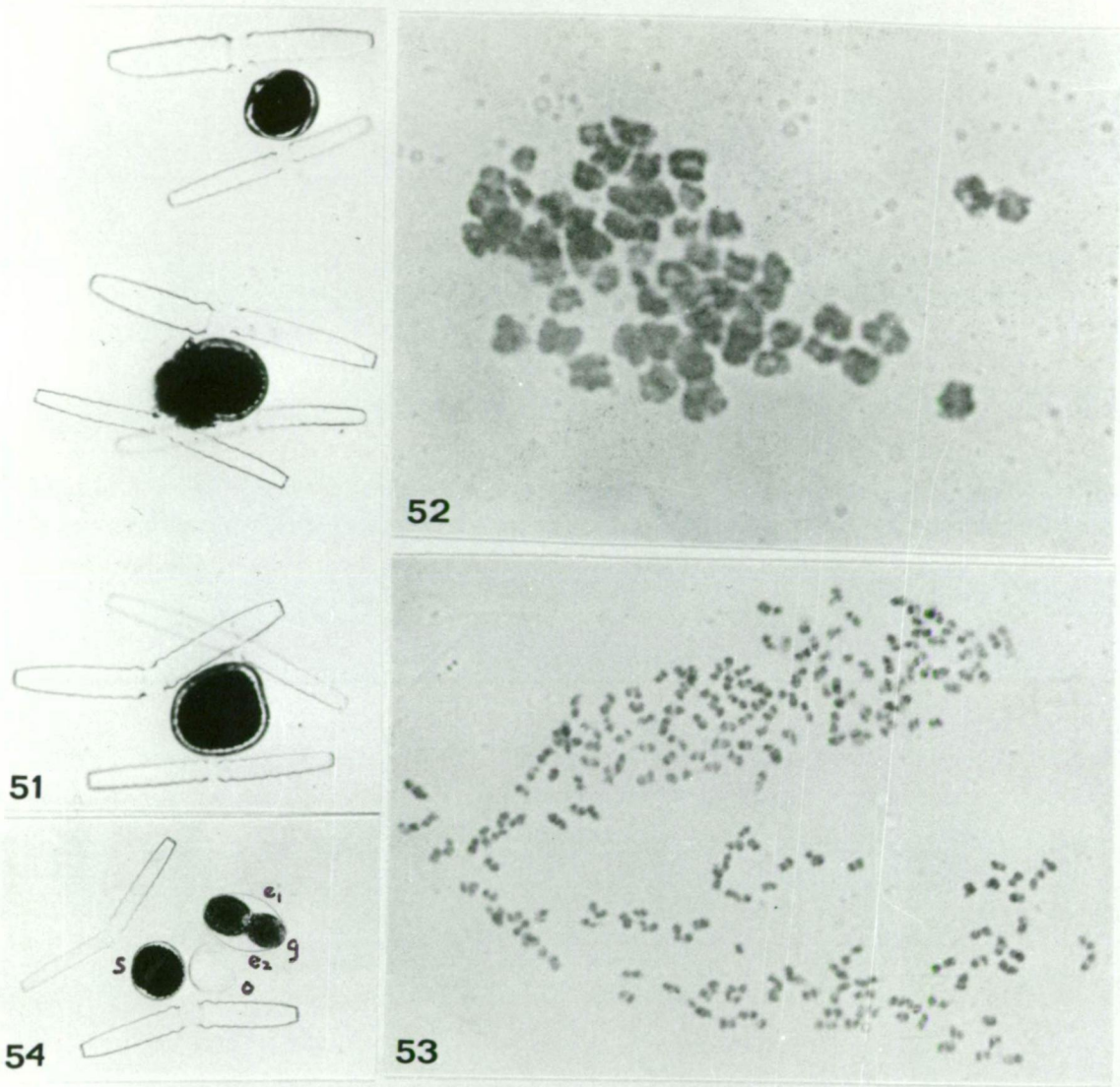


Fig. 51. Top to bottom; T 1 (slender cell) x N 1 zygospore; T 1 x N 1, one T 1 gamete has lysed; two T 1 by one N 1. x 125.
Fig. 52. Meiosis in T 1 x N 1. Metaphase I. x 2000.
Fig. 53a. Meiosis in T 1 x W 1. Late M II to early A I. x 1250.
Fig. 54. Single gone from T 1 x W 1 zygospore. g = gone, e_1, e_2 = endospores, o = operculum, s = exospore and mesospore. x 100.

N 1 or N 2 crosses (Fig. 51). In one T 1 x N 1 cross, conjugations involving two T 1 cells with one N 1 cell outnumbered simple conjugations 5 to 3. However, only 1 in 5 of the multiple conjugations was successful. In the rest one of the T 1 gametes lysed.

Germination and Meiosis.

T 1 x W 1 zygospores germinate at about diakinesis to metaphase I. A definite metaphase plate is formed. About 52 bivalents were counted in one metaphase I (Fig. 52).

Figures 53a, 53b show the two late metaphase II to early anaphase II in a vesicle. A total of 202 individual chromosomes can be counted. A clear central region is evident in most of the chromosomes.

Since only bivalents were observed (Fig. 52) it is reasonable to assume that T 1 and W 1 have very nearly if not an identical chromosome number of n = 51.

A single product is formed (Fig. 54). The F_1s are intermediate in size and shape between the parents.

Only a few germinations were observed in T 1 x N 1 or N 2 zygospores. These develop like those of T 1 x W 1. No meiotic details are available.

(i). Diploidy.

Large cells arose spontaneously in the T 1 culture. A clone T 1 2n maintaining the large cell-size was established. T 1 2n cells are significantly larger than normal (Fig. 48d; Table 5). Each semicell has up to 22 undulations and 9-(10.7)-12 apical tubercles.

Fig. 53b. Interpretation of 53a. 202 individual chromosomes can be counted.

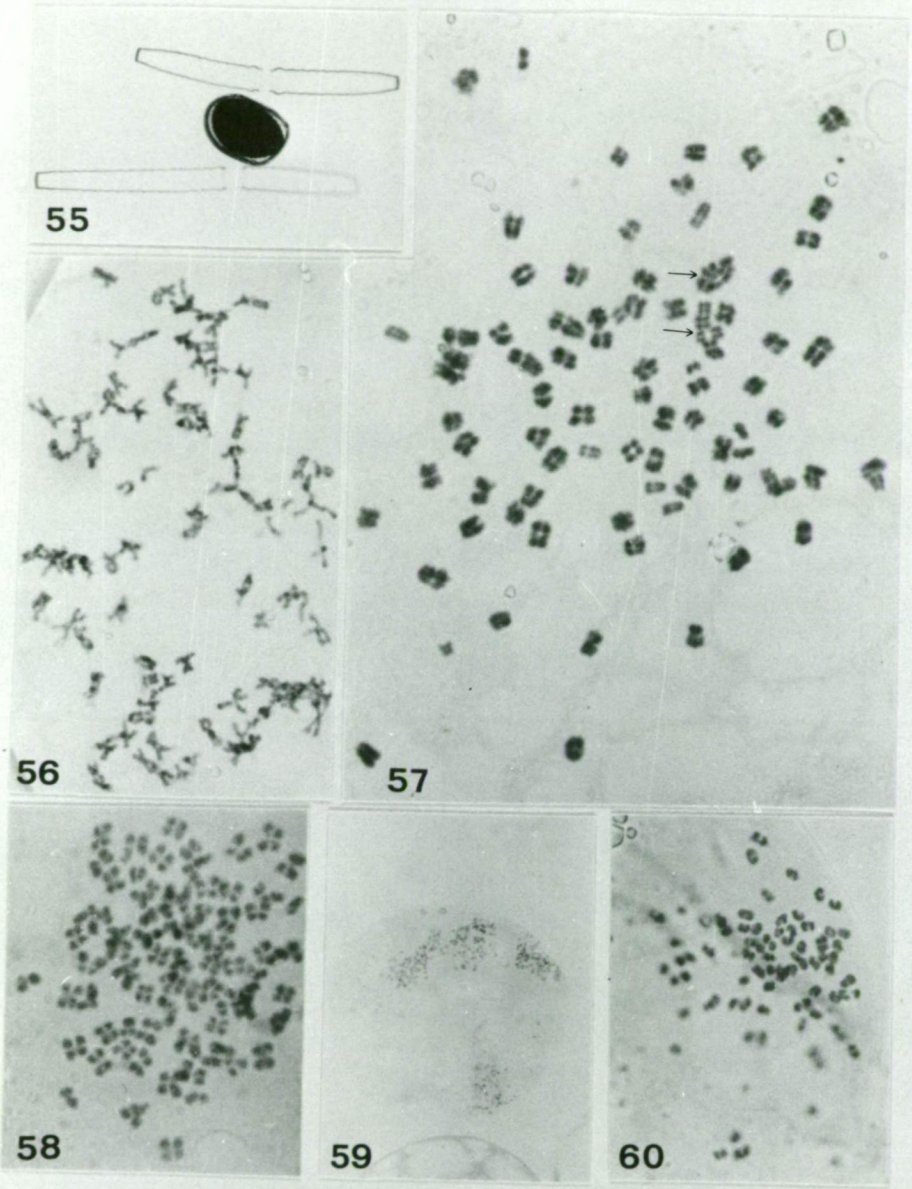


Fig. 55. T 1 2n (bottom cell) by W 1 zygospore. x 100
Figs. 56 - 60. Meiosis in T 1 2n x W 1.
Fig. 56. Leptotene with some stickiness. x 800.
Fig. 57. Metaphase I with unusual chromosomes (arrows). x1250.
Fig. 58. Metaphase II. Almost all the chromatids possess a clear central area. x 1250.
Fig. 59. Anaphase II. x 250.
Fig. 60. A single anaphase II group. Note the clear central area in most of the chromatids. x 1000.

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T 1 2n will form zygospores with W 1 and N 1 - 2 (Fig. 55). Pairing behaviour and zygospore characteristics are identical to T 1 by W 1 crosses.

One to two month old T 1 2n by W 1 zygospore germinate 2 - 4 days after immersion in fresh medium. Germination is usually enhanced when the drying-immersion process is repeated.

Some stickiness and probably multi-valents were observed in a leptotene preparation (Fig. 56). However, by metaphase I this has more or less disappeared (Fig. 57a, 57b). Some of the metaphase I chromosomes look unusual. A few are probably univalents and tri-valents. Others (arrows) defy interpretation. Estimates of three different metaphases I gave the equivalent of 76 , 81 , and 82 bivalents respectively.

The single early anaphase I observed was asynchronous, caused perhaps by the early movement of unpaired univalents to the poles.

Metaphase II chromosomes are organised into definite metaphase plates. In Figure 58 the chromosomes are mostly in pairs, some may be seen as individual rods, nearly all showing a median to sub-median constriction or clear area.

Figure 59 shows an anaphase II. The individual chromosomes are rod-like, most of which again show a clear central area. One of the groups contains about 78 chromosomes (Fig. 60a, 60b). The others have approximately the same number.

Assuming that T 1 2n is an exact diploid of T 1, then the difference between the number of pairs of chromosomes observed in metaphases II of T 1 x W 1 and T 1 2n x W 1 would be the chromosome

P. coroniferum

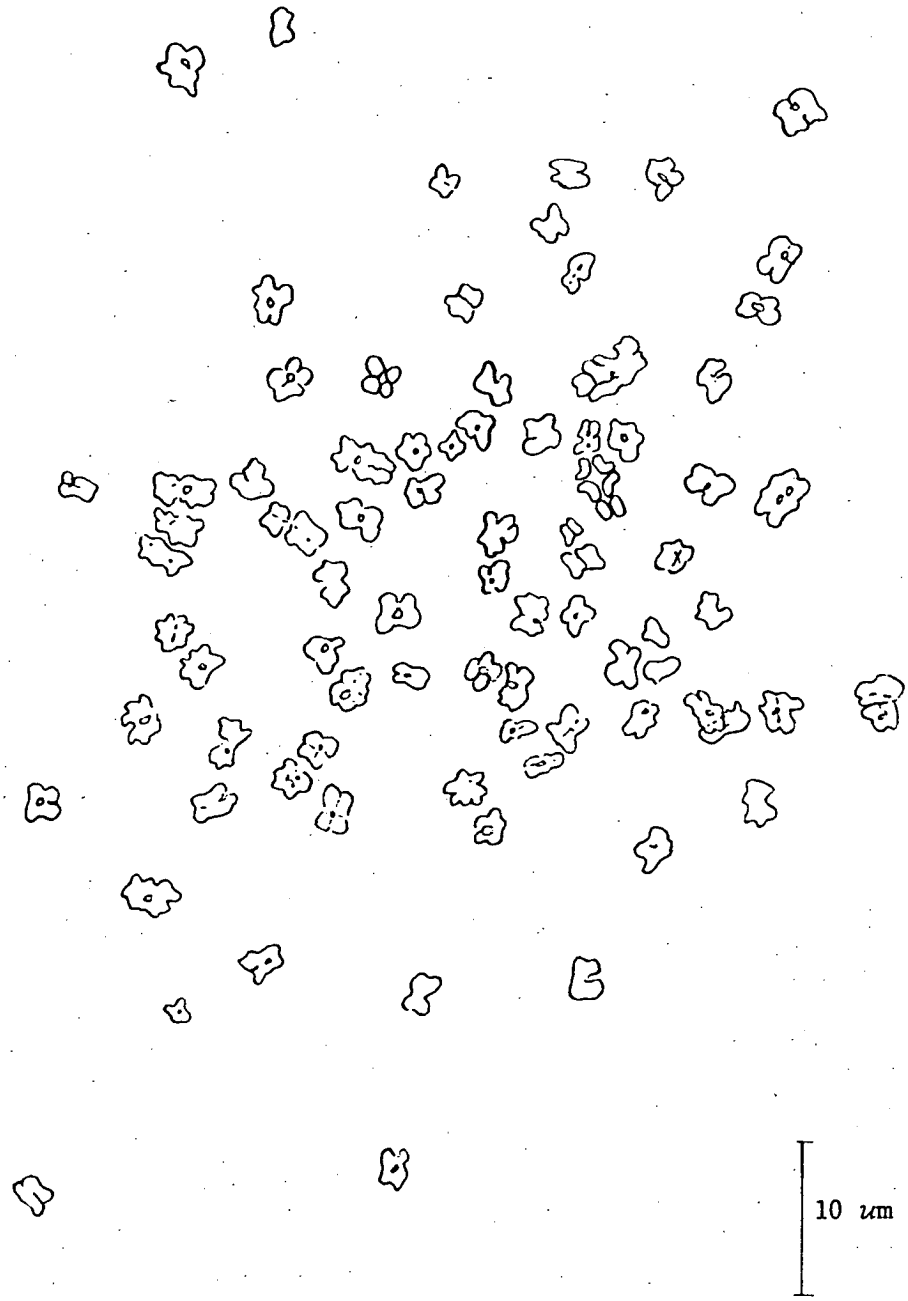

Fig. 57b. Interpretation of 57a.

P. coroniferum

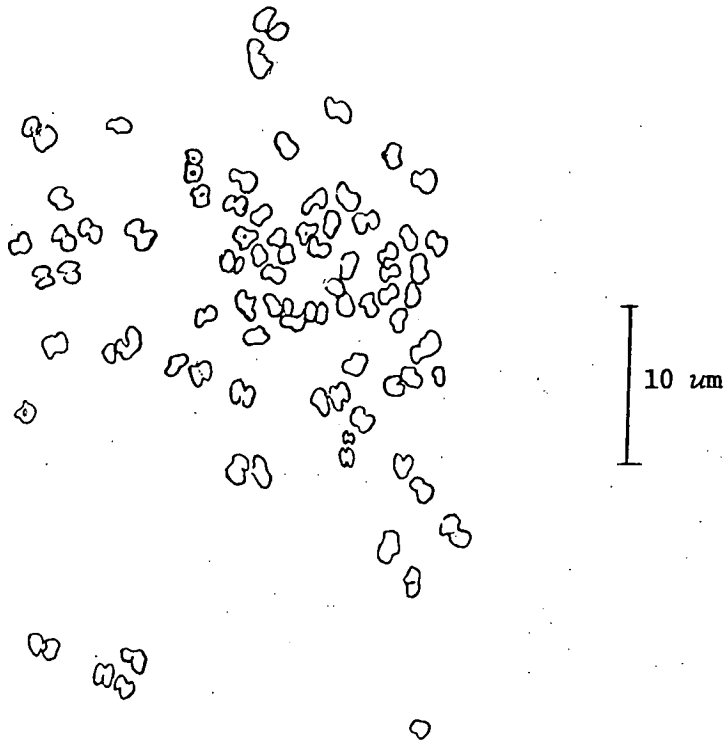

Fig. 60b. Interpretation of Fig. 60a.

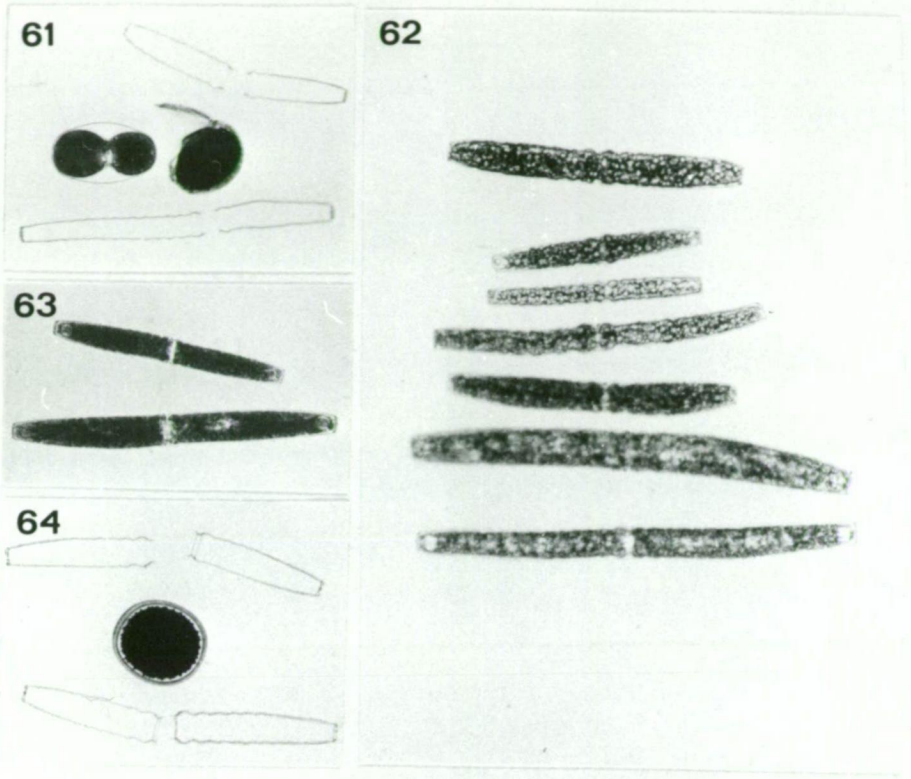

Fig. 61. T 1 2n x W 1. Single germination product. x 100.

Fig. 62. T 1 2n x W 1. F_1s, flanked by the parents. x 125.

Fig. 63. N 1. Haploid and large, presumed diploid, cells. x 100.

Fig. 64. Zygospore of Pine Lake *P. coroniferum*. x 125.

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number for T 1. 202 chromosomes, or 101 pairs, were observed in a T 1 x W 1 (Figs. 53a, 53b) and 76 x 2, or 152 pairs in a T 1 2n x W 1 (Figs. 60a, 60b). Thus T 1 has n = 51, agreeing with the previous estimate.

Three of the nuclei abort becoming round and stain deeply. A single product is formed (Fig. 61).

Certainly the most interesting result from a T 1 2n x W 1 cross is the large range in shape and size in the F_1s. Five of the more distinctive types flanked by the parents are shown in Fig. 62. Most of these can be related back to the parents. For example, the largest one appears to have inherited the length from one parent and the width from the other. The small cells are even more intriguing. One of them looks exactly like a cell from the T 1 clone! The exact proportions of these cell types in the F_1s is unknown. The majority of the F_1s assume a shape intermediate between the parents.

T 1 2n x N 1 - 2 zygospores are difficult to germinate.

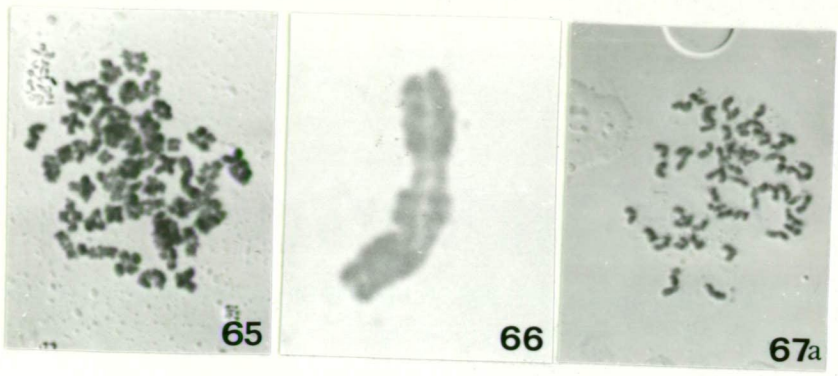
Diploid N 2.

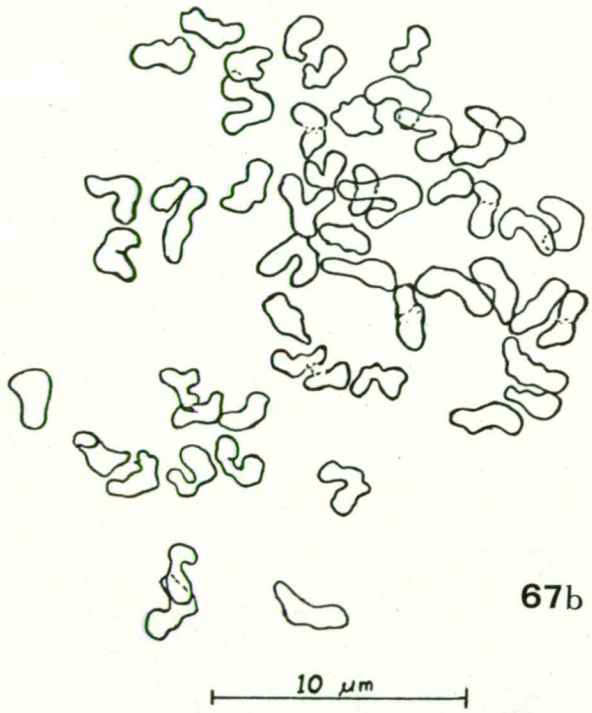
A presumed diploid clone of N 2 was recently isolated. Cells of the clone are significantly larger than normal (Fig. 63; Table 5). Each semicell has 11 - 14 apical tubercles.

(ii). Pine Lake *P. coroniferum.*

The recent Pine Lake sample contained many *P. coroniferum* cells. Zygospores were formed when the sample was subjected to culture conditions (Fig. 64).

The zygospores were germinated successfully. About 50 bivalents





Figs. 65 - 67. Meiosis in Pine Lake *P. coroniferum*.

Fig. 65. Metaphase I. About 50 bivalents. x 1100.

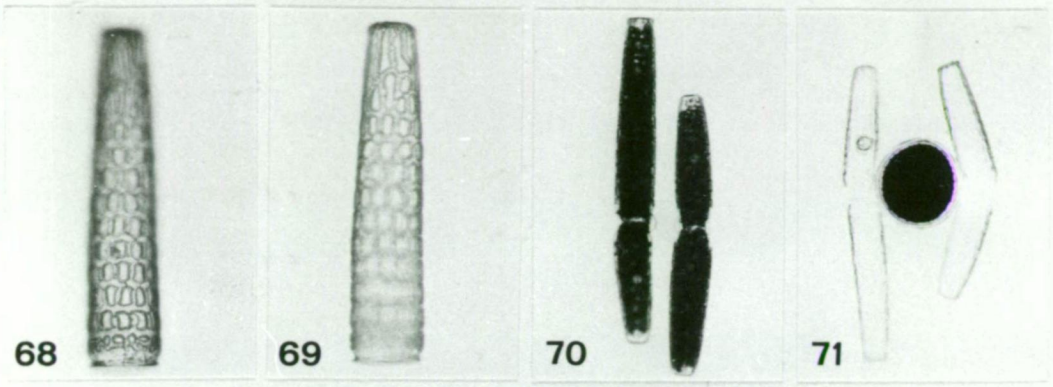
Fig. 66. Chain of bivalents. x 3200.

Fig. 67a. Anaphase II. x 1100.

Fig. 67b. Interpretation of Fig. 67a. About 53 chromosomes.

were observed in a metaphase I (Fig. 65). A small amount of stickiness was also observed. Figure 66 shows what appears to be a chain of three bivalents. An estimate of about 53 chromosomes was made of an anaphase II (Figs. 67a, 67b). The F_1 retain the shape of the parents.

Clones were isolated by separating pairs of conjugants from the raw sample and culturing the cells individually. The clones are heterothallic and have formed zygospores with both T 1 and W 1.



Figs. 68, 69. Air-dried empty semicell at two focal levels. x 250.

Fig. 70. Conjugation. Production of papillae. x 125.

Fig. 71. Mature zygospore. x 125.

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3. *Pleurotaenium verrucosum* (Bail.) Lund.

Taxonomy.

The plants were observed in a Queensland sample collected by Dr. P. W. Cook. Each semicell has a tesselated appearance (Figs. 68 - 69), lacks a basal inflation and tapers towards an apex adorned with about 10 conical to pointed tubercles.

There is little doubt that the plants are *P. verrucosum*. Whether they are more closely identifiable with one of its varieties is debatable. Scott & Prescott (1958) described "a form, from Yirrkalla, Queensland, in which the margins are very slightly, if at all, attenuated toward the apices which bear a ring of prominent, sharply pointed teeth". Their Fig. 2 No. 5 shows only a very slight basal inflation. Krieger (1937, Pl. 51, Fig. 3) depicted *P. verrucosum* from Java, Australia, etc., as having a basal inflation, more or less parallel sides and 5 apical tubercles. His var. *coronatum* (Pl. 51, Fig. 4) from South Sumatra lacks a basal inflation and is tapered towards an apex bearing 8 tubercles. In both of Krieger's figures the tubercles are conic to rounded and it appears that the number of tubercles cited are actually those observed side on, i.e. only on one half of the apex. Another one of Krieger's varieties, var. *bulbosum* (Pl. 51, Fig. 5) is very slightly tapered towards an apex bearing 4 sharp-pointed tubercles.

Conjugation.

The sample was subjected to culture conditions. A few pairs of cells conjugated (Figs. 70 - 71). The conjugation process and

zygospore characteristics do not differ significantly from those described for *Pleurotaenium* species. The operculum is smooth.

Attempts at culturing the cells or germinating the few zygospores were unsuccessful.

4. *Pleurotaenium trabecula* var. *mediolaeve* (Playf.) Krieg.

The process of conjugation and zygospore germination in *P. trabecula* var. *mediolaeve* were described previously (Ling & Tyler, 1972a, 1972b) and follows the pattern for *Pleurotaenium* species generally. The origin of strains and their mating types are shown in Table 6.

(i). Meiosis.

Newly released vesicles are usually at diakinesis or early metaphase. Fig. 72 is an interpretation of a diplotene/diakinesis stage. In almost all the bivalents, especially the one in Fig. 73, the individual chromatids are clearly visible. The longest of the bivalents (arrow) appears to have three chiasmata. A total of about 52 bivalents can be counted. The little chromosome (double arrow) is probably a tiny bivalent.

Towards metaphase I the bivalents condense and move close together (Fig. 74). In Fig. 75 about 52 bivalents can again be counted. The long bivalent (arrow) is also evident. Fig. 75 came from a BW 1 x P 1 cross. Fig. 72 came from P 1 x F_1 4, a backcross to one of the parents.

Anaphase I was observed twice. They were both synchronous except for a single laggard in one of them (Fig. 76). There is no interphase, the chromosomes passing directly into metaphase II.

Metaphase II chromosomes show so much stickiness that accurate counts could not be made. Nevertheless, not all of the chromatid and chromatid pairs are stuck together and in each chromatid a

Table 6. Source and mating type of strains of *P. trabecula* var. *mediolaeve*.

Strain	Source	Mating type
BW 1	Brownwater Lagoon, Tas.	+
P 1	Pond, Strickland Ave., Hobart, Tas.	-
F_1 4	F_1 of BW 1 x P 1	+
F_1 1	F_1 from a triple conjugation zygospore of a BW 1 x P 1	-
BW 1 2n	From BW 1 in culture	+

P. trabecula var. *mediolaeve*

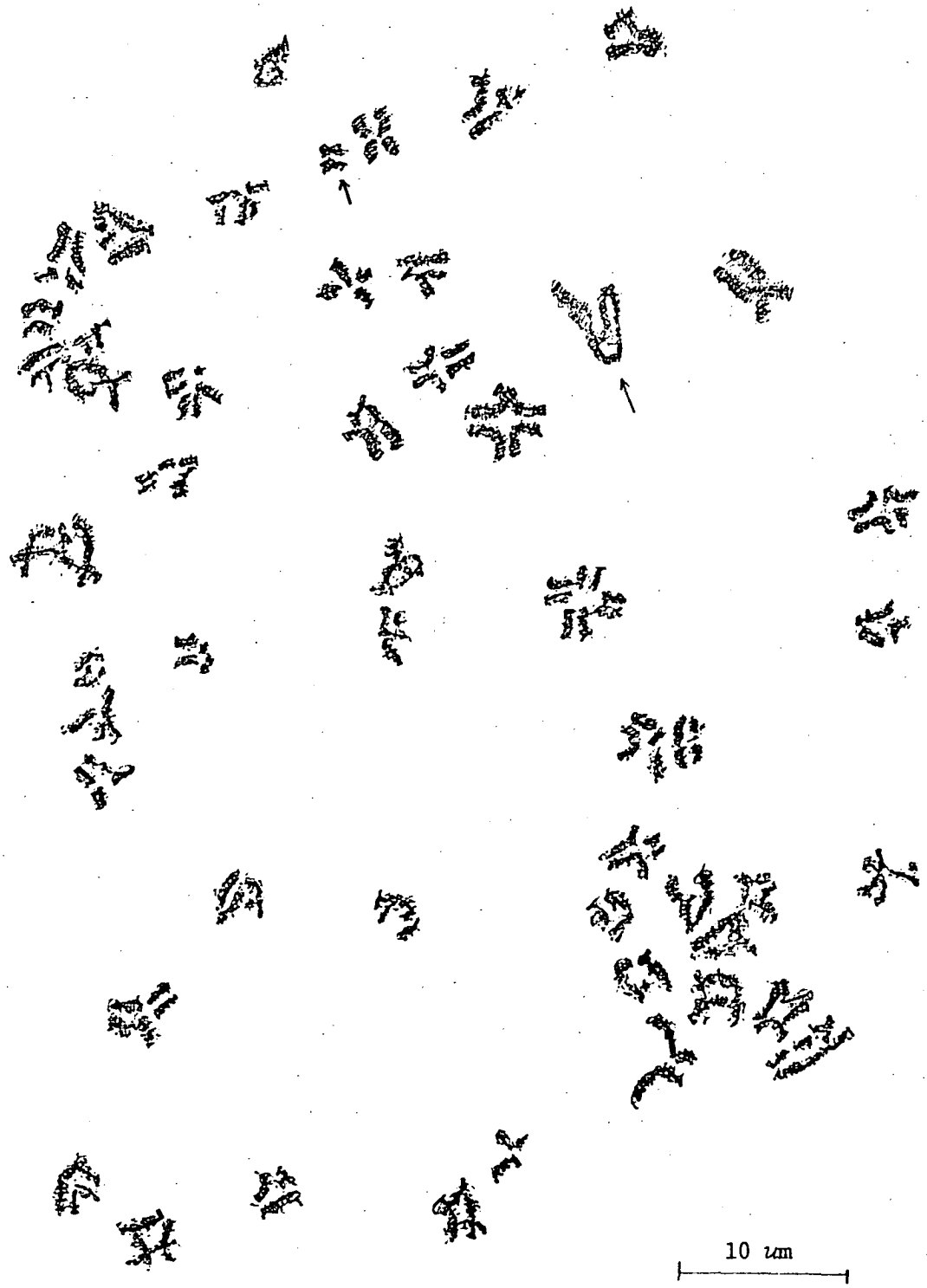
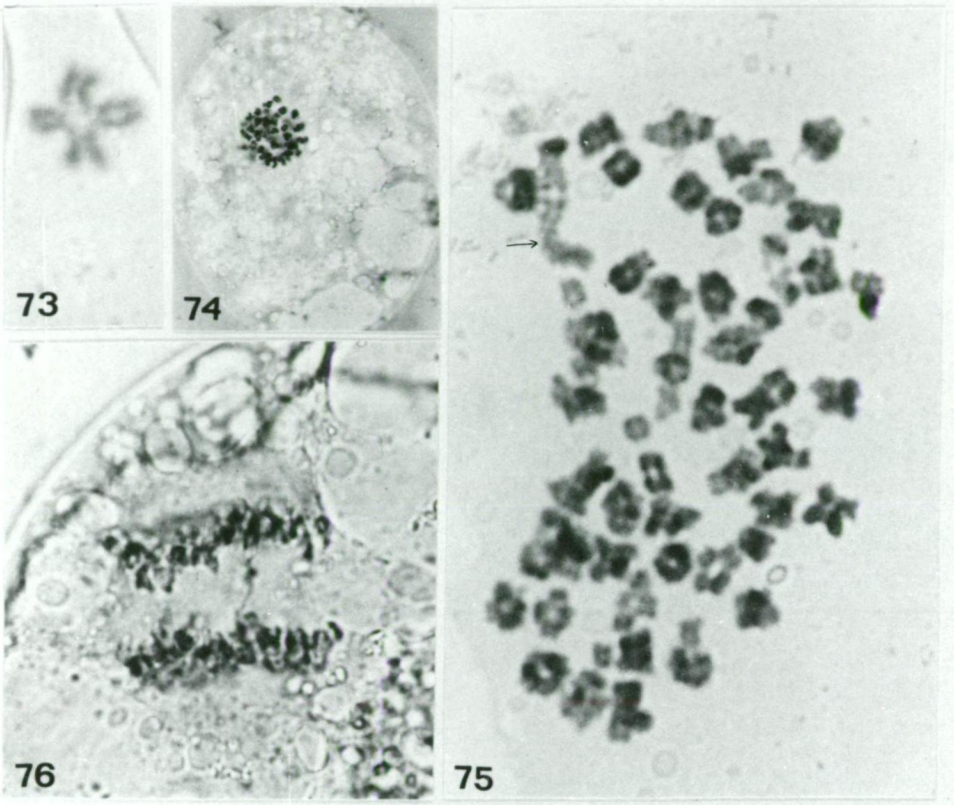


Fig. 72. Interpretation of a diplotene/diakinesis. Arrows point to a long and a small bivalent.



Figs. 73 - 76. Meiosis in *P. trabecula* var. *mediolaeve*.

Fig. 73. Cross bivalent with each chromatid clearly visible. x 3000.

Fig. 74. Vesicle with early metaphase I plate. x 250.

Fig. 75. Metaphase I. Note long bivalent (arrow). x 2000.

Fig. 76. Synchronous anaphase I with a probable chromosome bridge. x 1300.

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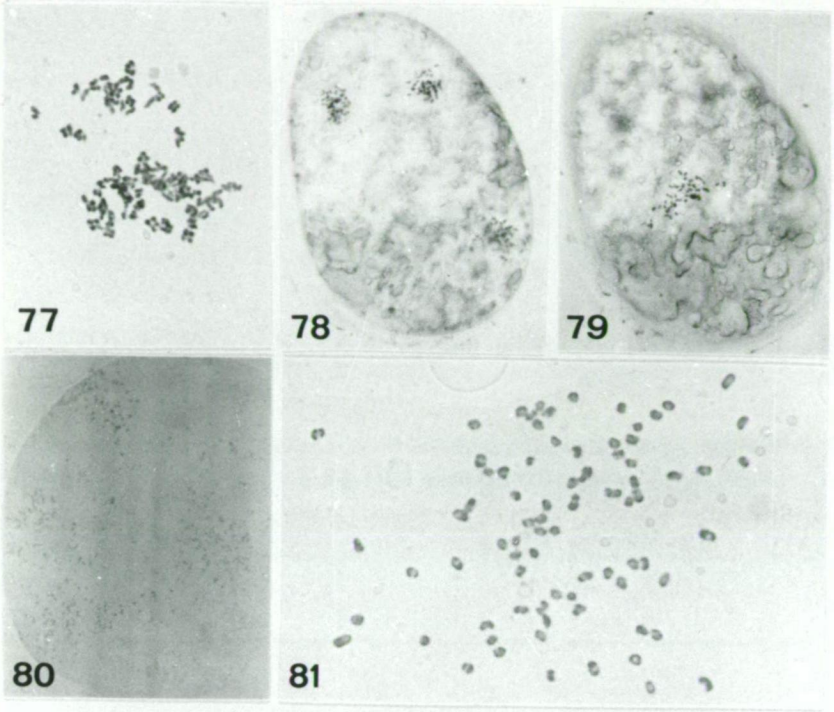

Fig. 77. Metaphase II with stickiness. x 750.

Figs. 78 - 79. Vesicle at late anaphase II. Two focal levels. x 300.

Figs. 80 - 81. Triploid meiosis.

Fig. 80. Anaphase II. x 250.

Fig. 81. Enlargement of group (i) of the anaphase II groups in Fig. 80. 78 chromosomes can be counted. x 1000.

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median constriction is usually evident (Fig. 77).

Figs. 78 and 79 show a late anaphase II. There is slight stickiness. Three of the nuclei abort. A single gone is formed.

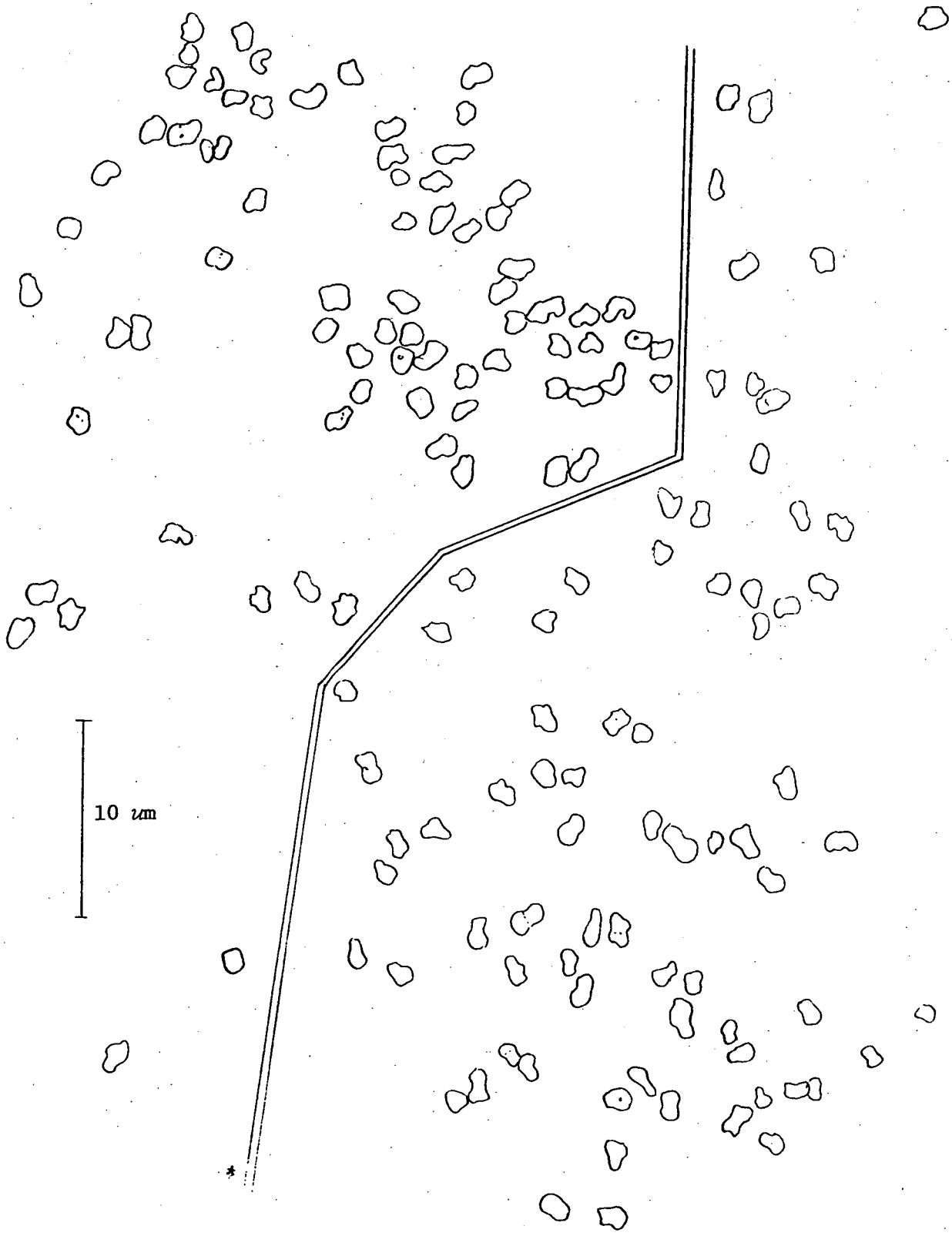
(ii). Polyploidy.

Triple conjugation product.

Clone F_1 1 arose from a gone produced by a triple conjugation zygospore (Ling & Tyler, 1972b). The semicells have 16 - 18 (normal semicells have 11 - 16) apical tubercles and are significantly larger than normal.

F_1 1 is minus in mating type and will produce large numbers of zygospores with BW 1. Like the diploid zygospores these germinate at diakinesis to metaphase I. Metaphase I chromosomes consist mainly of bivalents with a few presumptive uni-, tri-, and quadrivalents.

The majority of one batch of vesicles were at anaphase II. Fig. 80 shows one such vesicle. There is very little, if any, stickiness. Most of the chromosomes clearly show a median lighter area or constriction (Fig. 81). 75, 75, 77 and 78 chromosomes respectively were counted in the four groups (Figs. 81 - 83). Estimates of several other anaphase II groups gave counts ranging from 72 to 80, but in each vesicle the total is about 300 - 310. Of the 305 (75 + 75 + 77 + 78) chromosomes about 104 would have come from the haploid BW 1 thus giving an n of about 100 for F_1 1, almost double the haploid complement. This is rather surprising considering that F_1 1 came from a triple conjugation zygospore. One would expect



Figs. 82 - 83. Enlargement of the four groups of chromosomes from a late anaphase II (Fig. 80).

Fig. 82. 1st and 2nd groups each containing 75 chromosomes.

* *Groups are drawn close together to fit page.*

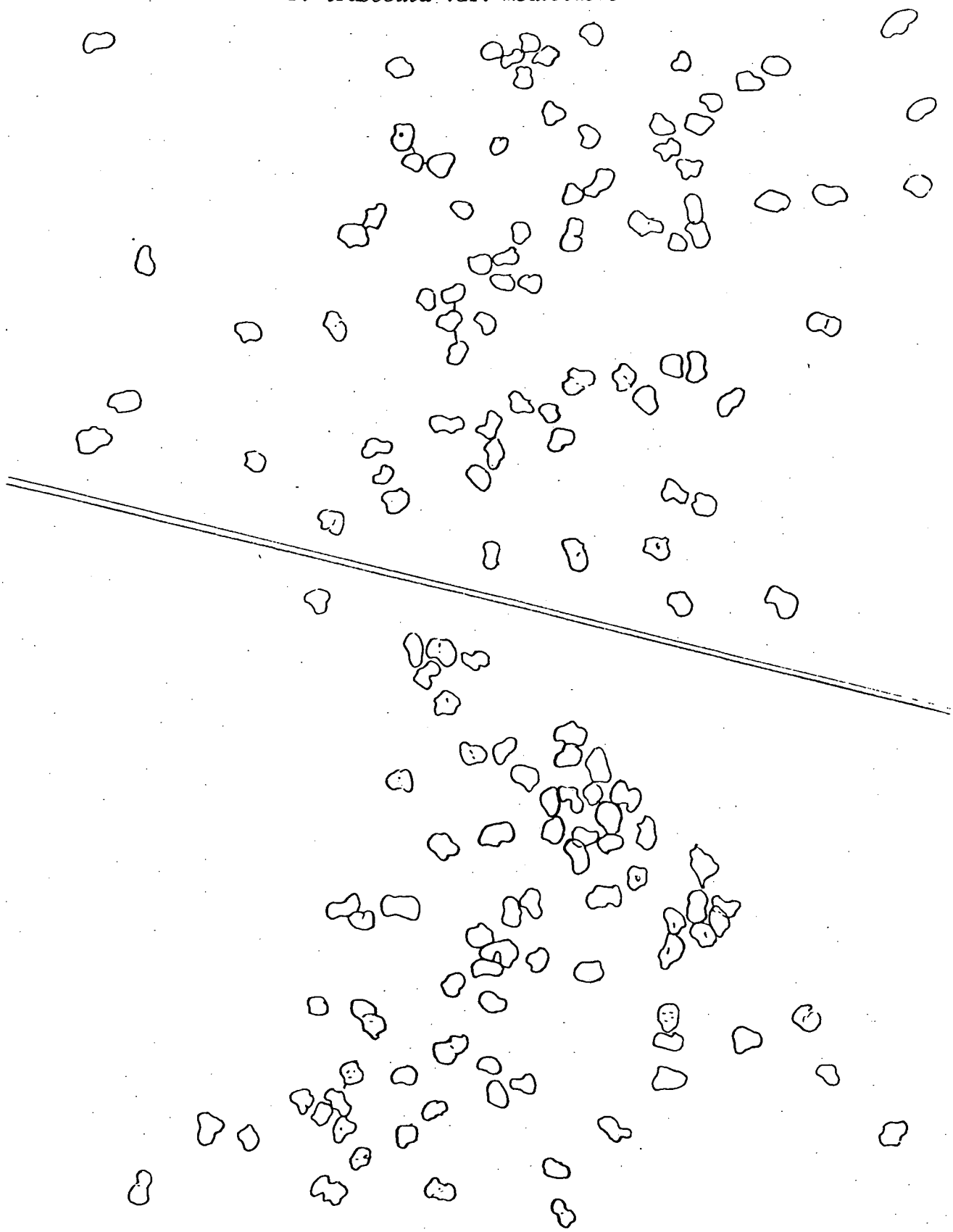
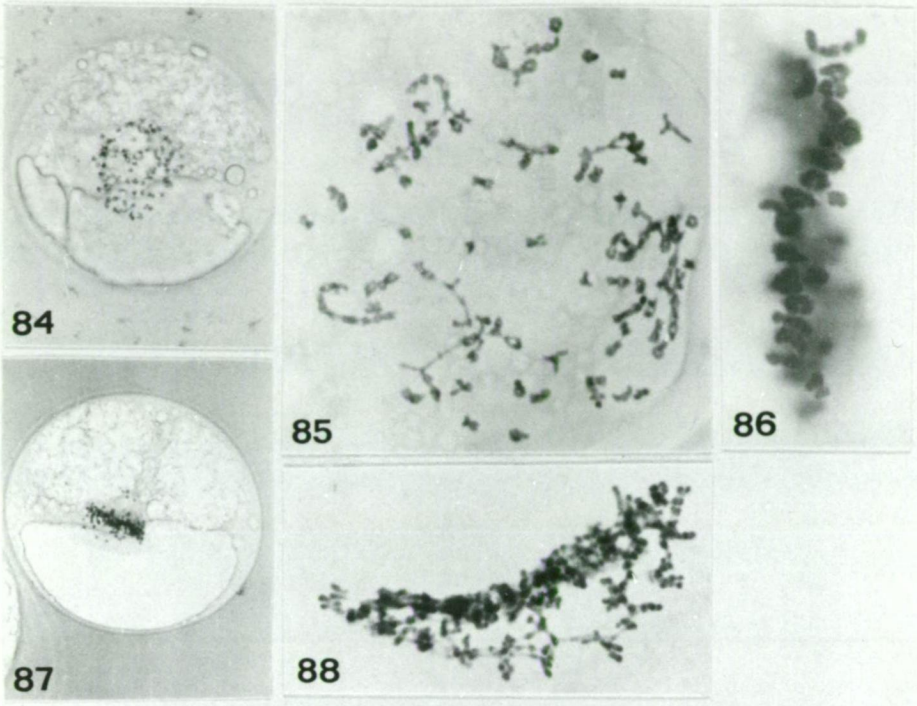


Fig. 83. 3rd (Fig. 81) and 4th groups.



Figs. 84 - 88. Tetraploid meiosis.

Fig. 84. Vesicle at early metaphase I. The chromosomes have not yet moved close together. x 250.

Fig. 85. Diakinesis, with much stickiness. x 750.

Fig. 86. Metaphase I, equatorial view. x 1300.

Fig. 87. Vesicle with asynchronous anaphase I. x 250.

Fig. 88. Metaphase II with extreme stickiness. x 1000.

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it to be 1½-ploid with about 78 chromosomes. It may have received more than its fair share of chromosomes, but the fairly even distribution of chromosomes at anaphase II in a BW 1 x F_1 1 cross suggest that this is unlikely. The most likely explanation is that one of the triple conjugation cells was a binucleate or a diploid with a reduced size.

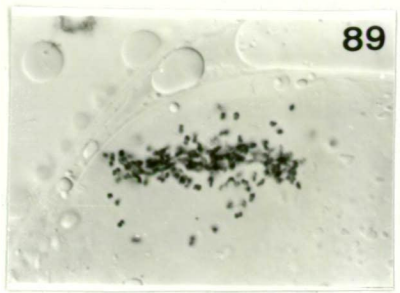
BW 1 x F_1 1 crosses are fertile. The F_1s are intermediate in size and interfertile. An F_1 clone was backcrossed successfully to BW 1.

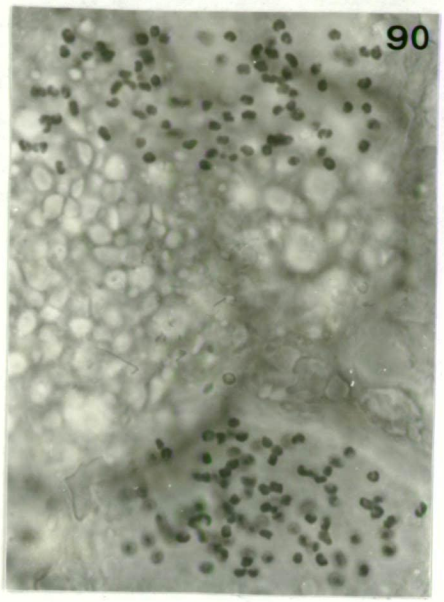
Diploid *P. trabecula* var. *mediolaeve*.

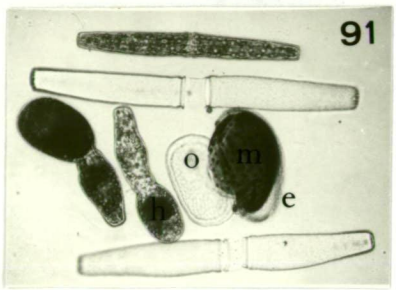
BW 1 2n originated as a large cell from BW 1. BW 1 2n will cross with F_1 1. Zygospore germination does not differ substantially from that of haploids. Fig. 84 shows an early metaphase I. The chromosomes have not begun to move close together. Fig. 85, another early metaphase I, paints a completely different picture. Many of the chromosomes, up to four at a time, are joined end to end forming chains. In Fig. 86 the chromosomes have moved closer together forming a plate.

An early anaphase I observed was asynchronous, some of the half-bivalents moving polewards while most of the chromosomes are still at the equator (Fig. 87).

There was not a single metaphase II which did not show some stickiness. In some cases the stickiness is extreme (Fig. 88). It is amazing how the chromatids manage to disentangle themselves (Fig. 89) and spread out as individual short rods by anaphase II (Fig. 90). Here again, the individual chromosomes have a light staining or







Figs. 89 - 90. Tetraploid meiosis.

Fig. 89. Asynchronous anaphase II. x 600.

Fig. 90. Anaphase II. Arrows point to chromosomes with constricted or clear central areas. x 1000.

Fig. 91. First gonal division of product from a tetraploid zygospore. Haploid cell at top is included for comparison. e = exospore, m = mammillate mesospore, o = operculum, h = haematochrome. x 250.

constricted middle. Estimates of chromosome numbers at anaphase II are consistent with the assumption that BW 1 2n is a diploid. The F_1 maintain the large size of the parents (Fig. 91).

Two other presumed diploid clones were isolated from F_1 clones of BW 1 x P 1.

P. excelsum

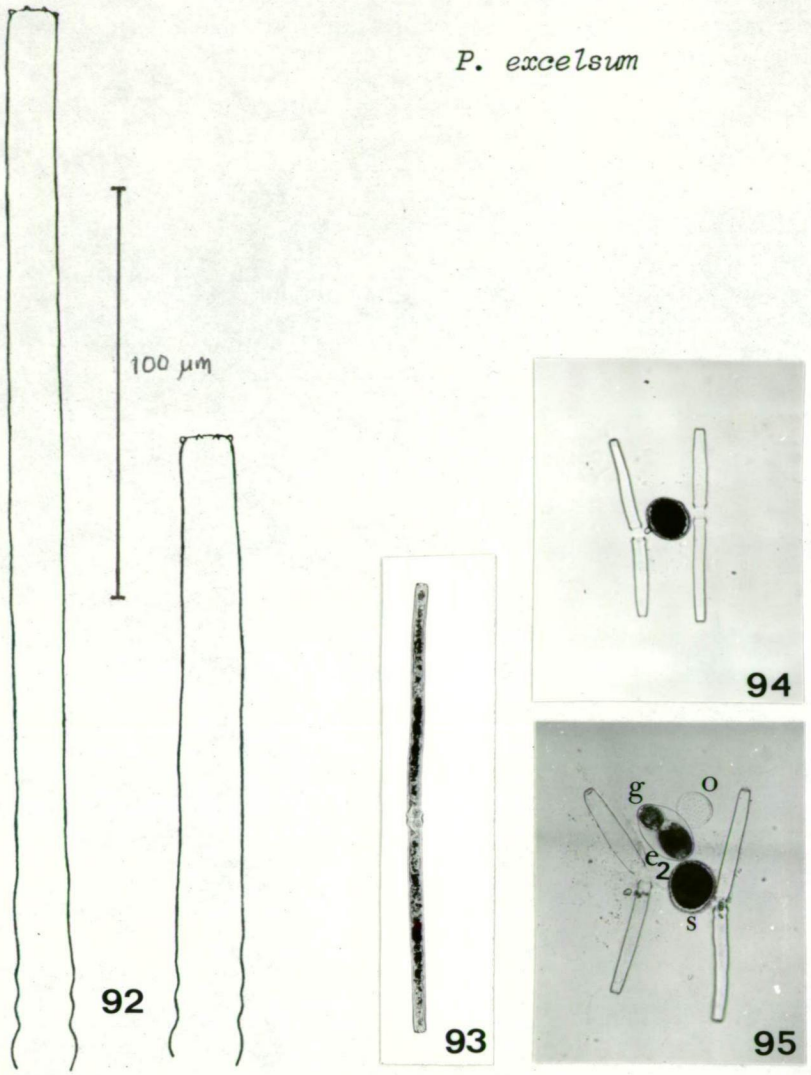


Fig. 92. Semicells from Yan Yean Reservoir.

Fig. 93. Cell from Yan Yean Reservoir. x 125.

Fig. 94. Zygospore. x 125.

Fig. 95. Single gone from zygospore. g = gone, s = exospore and mesospore, o = operculum, e_2 = endospore 2. Endospore 1 present but not evident here. x 125.

5. *Pleurotaenium excelsum* (Turn.) Gutw.

Taxonomy.

The plants (Figs. 92, 93) agree with descriptions and figures of *P. excelsum* given by Krieger (1937, Figs. 8, 9) and Scott & Prescott (1958, Fig. 2, No. 3).

Cells of *P. excelsum* are long and slender. Each semicell has a distinct basal inflation and 2 - 3 slight undulations immediately above it. The apex is ornamented with a ring of 6 - (9.2) - 11 small, conical tubercles. Dimensions of some cells from Yan Yean Reservoir, Victoria, are:
343-(417)-495 x 16-(17.6)-19 x 13-(14.3)-15 x 10-(12)-13 *um*.

Two clones, one from Yan Yean the other from Tocumwal, New South Wales, were isolated. Cultured cells are about half as long as the longest of the wild cells. The apical tubercles are reduced or absent.

Sexual reproduction.

The two clones are opposite in mating type. Sexual reproduction follows the general pattern for *Pleurotaenium* species (Figs. 94, 95). The operculum is finely punctate. The F_1s are interfertile.

A small clone of large, presumably diploid cells, was isolated from the Yan Yean clone.

Attempts to cross *P. excelsum* with *Pleurotaenium* T_4 were unsuccessful.

Pleurotaenium T_4

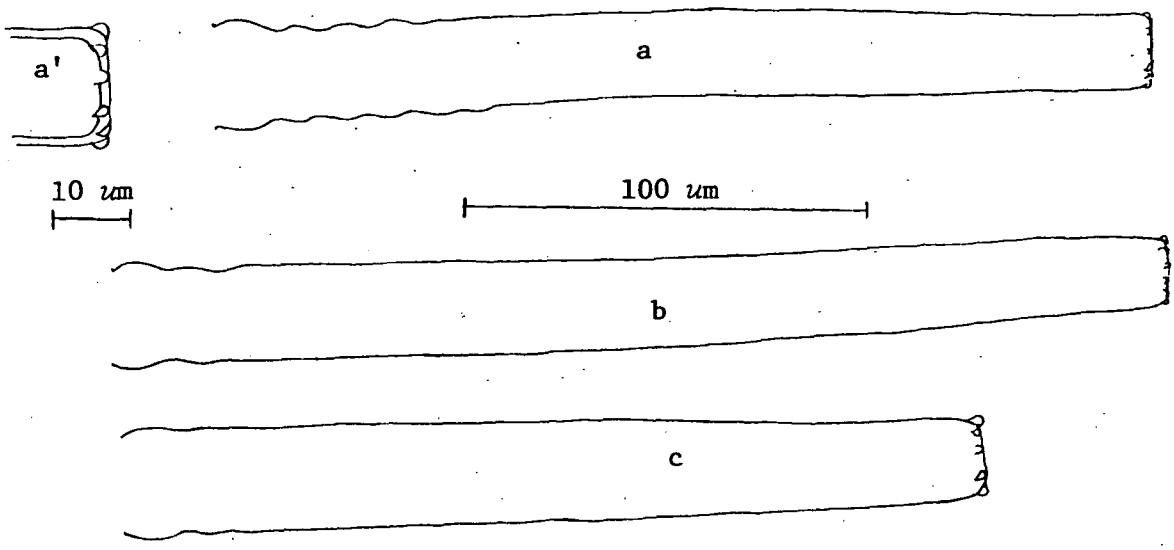


Fig. 96. Semicells from Yan Yean Reservoir (a) and Goulburn Weir (b, c).

Table 7. Dimensions, source and mating types of *Pleurotaenium* T_4 strains.

Haploid ex-culture: 236-(270)-304 x 17-(18)-20 x 13-(15)-16 x 10-(13)-14 *um*
Diploid ex-culture: 304-(368)-433 x 24-(26)-27 x 20-(22)-24 x 14-(16)-18 *um*

Strain	Source	Mating type
YY 1 - 3	Yan Yean Res., Vic.	-
YY 4	Yan Yean Res., Vic.	+
GW 1	Goulburn Weir, Vic.	-
GW 1 2n	From GW 1 in culture	-
F_1 2n 1	F_1 of YY 3 x YY 4	-
F_1 2n 5		+
F_1 2n 6		-
F_1 2n 7		+

No specific names were given to the following three *Pleurotaenium* species because of some confusion of identity and difficulty in making iconographic matches. A minor reason is that there appears to be an unusual and as yet unresolved relationship between the three species.

6. *Pleurotaenium* T_4.

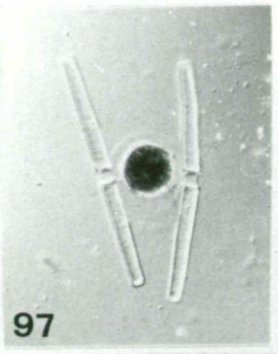
The cells are slender. Each semicell has from 1 - 4 undulations immediately above the basal inflation and bears from 11 - 14 conic to rounded apical granules (Fig. 96).

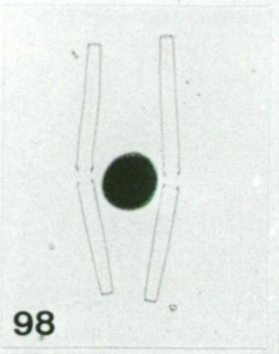
Four clones were isolated from Yan Yean Reservoir (YY 1 - 4) and one from Goulburn Weir (GW 1). Cultured cells are reduced in size (Table 7) and the apical granules are barely visible in side view, though in end view they are still distinct.

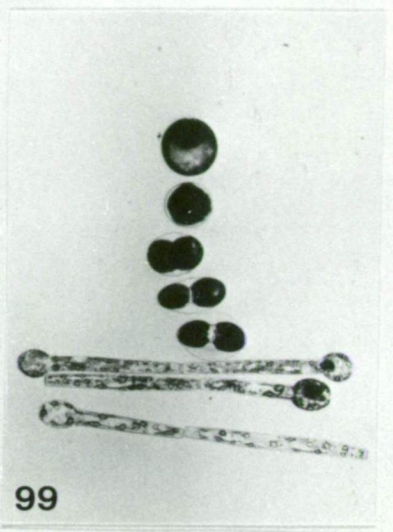
The clones are heterothallic and their mating types are listed in Table 7. The operculum is punctate. Sexual reproduction (Figs. 97 - 99) and meiosis does not differ significantly from that of other *Pleurotaenium* species. A count of 49 bivalents was obtained from a metaphase I (Fig. 100).

One of the germination vesicles produced an unusual gone (Fig. 101). Divisions took place at both isthmuses but attempts at isolating clones were unsuccessful. The gone is probably a binucleate, the result of the survival of two daughter nuclei instead of the normal one.

The F_1s are interfertile.







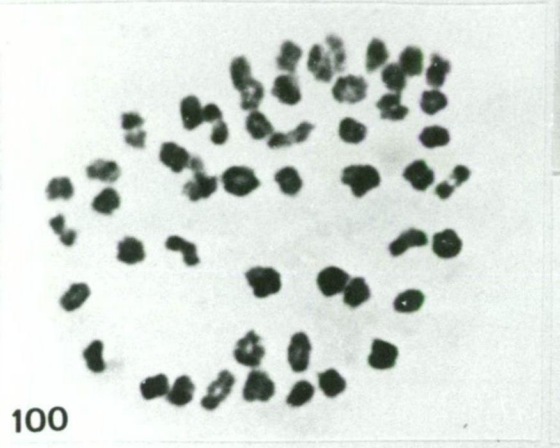


Fig. 97. Immature zygote surrounded by the conjugation vesicle. Gamete exit pore is visible in the lengthened isthmus of the cell on the left. x 125.

Fig. 98. Zygospore. x 125.

Fig. 99. Stages in the development of a single gone. From top to bottom; newly released vesicle with single chromatophore, vesicle with condensed cytoplasm, furrowing, single gone, gone starting to divide, first gonal division, second gonal divisions. x 100.

Fig. 100. Metaphase I. 49 bivalents can be counted. x 2000.

Fig. 101. Abnormal gone with two isthmuses. x 250.

7/18/95

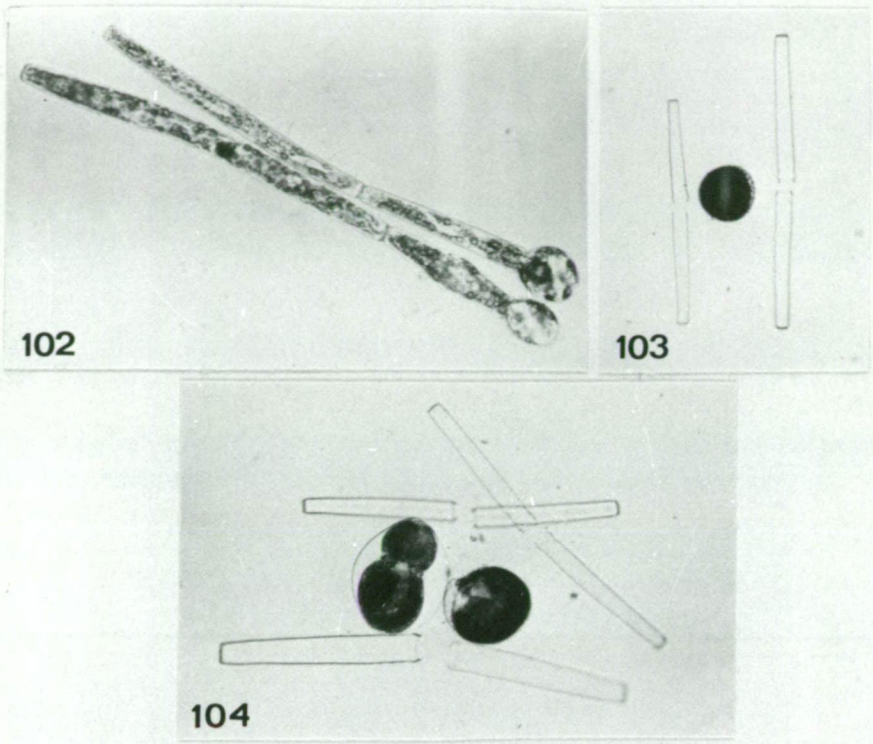


Fig. 102. Second gonal divisions, the lower cell producing a larger than normal cell. The cells are from two separate gones. x 150.

Fig. 103. Triploid zygospore. x 125.

Fig. 104. Tetraploid (n x n x 2n) zygospore germination. x 150.

96/2/LL

Diploidy.

Five presumed diploid clones were isolated; one from a large cell produced spontaneously in GW 1, three from large cells found in a mixed F_1 culture and the fifth from a germination product (Table 7). The germination product looked like any other gone but the new semicells produced in the first and subsequent divisions were larger than normal (Fig. 102).

In addition to their large size (Table 7) diploid cells have 16 - (17.5) - 19 apical tubercles.

The mating types of the diploid clones are listed in Table 7.

Sexual reproduction in diploid x haploid.

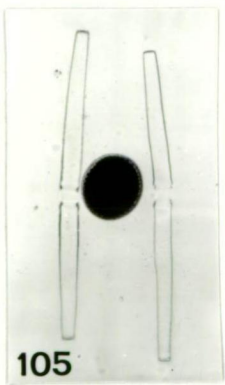
In a YY 3 x F_1 2n 5 cross, clumps of cells consisting of one F_1 2n 5 cell surrounded by two to four YY 3 cells were common. A large number of zygospores were formed. Out of 60 randomly selected zygospores, 23 were formed from n x 2n (Fig. 103); 26 involved n x n x 2n but the 2n cell formed a zygospore with only one of the n cells, the other gamete lysing; and 11 were formed from multiple conjugations, n x n x 2n.

The zygospores were germinated successfully. Nearly all the vesicles reached the gonal stage. A large number then rupture during division. Many of the survivors produced cells the size of the diploid parent and there is little doubt that these have come from the tetraploid (n x n x 2n) zygospores (Fig. 104). Cells of intermediate size, presumably from the triploid zygospores, are much fewer in number.

Tetraploid meiosis.

Tetraploid zygospores (Fig. 105) formed by F_1 2n 6 x F_1 2n 7 appear to germinate as easily as diploid ones. Fig. 106 is a metaphase I from one of the germination vesicles. Chromosome associations consist almost entirely of bivalents though possibly an odd univalent, trivalent and quadrivalent are also present. A total of about 100 "bivalents" can be counted proving that the large cells are indeed diploids.

The F_1s are all large and interfertile.



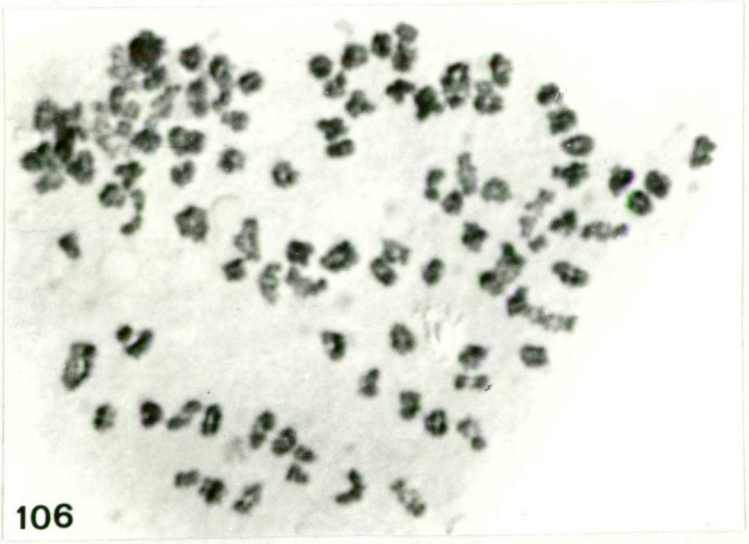


Fig. 105. Tetraploid (2n x 2n) zygospore. x 100.

Fig. 106. Tetraploid meiosis. Metaphase I, approximately 100 "bivalents" can be counted. x 2000.

Pleurotaenium T_3

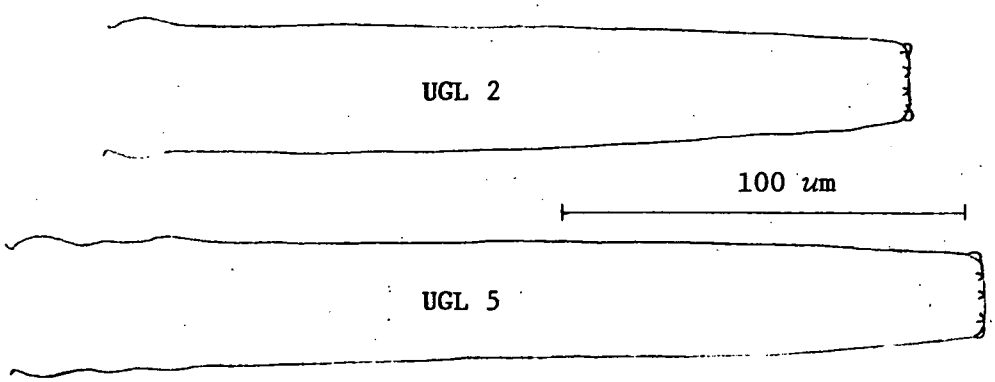


Fig. 107. Wild type semicells of *Pleurotaenium* T_3.

Table 8. Source and mating type of *Pleurotaenium* T_3 clones.

Source	Mating type +	Mating type -
Victoria		
Upper Gunbower Lagoon	UGL 2 UGL 5	UGL 13
Glenmaggie Res'r	GM 1 GM 2	GM 3
Yan Yean Res'r		YY 2 YY 3 YY 4 YY 5
Wartook Res'r	WK 1	
Tasmania		
Lake Sorell		LS 1

7. *Pleurotaenium* T_3.

Taxonomy.

Clones were isolated from several Victorian and one Tasmanian localities (Table 8). The semicells have straight to slightly undulate sides above the basal inflation and 8 - 12 apical beads or granules (Fig. 107). Dimensions of cells from Upper Gunbower Lagoon are:

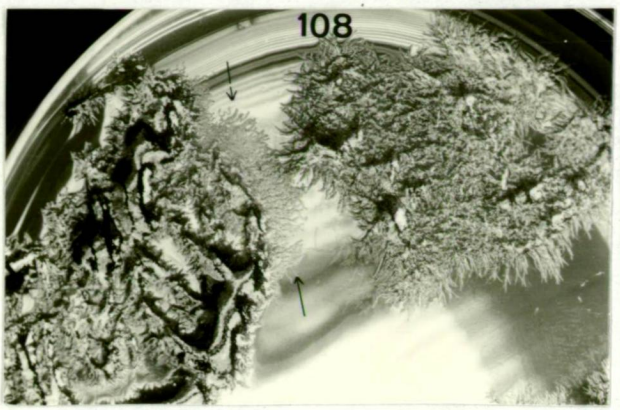
l.	*b.*	*i.*	*a.*
350-(390)-460 x	27-(34)-38 x	25-(28)-30 x	15-(19)-21 m.

The plants are practically indistinguishable from *P. trabecula* var. *mediolaeve* and were initially isolated as such. *P. trabecula* var. *mediolaeve* has 11 - 16 apical beads (Ling & Tyler, 1972a). Playfair (1907) originally described *P. trabecula* var. *mediolaeve* as having 10 - 12 apical rugae. With a much wider known distribution and 8 - 12 apical beads it thus appears that *Pleurotaenium* T_3 fits Playfair's description better than the strains described by Ling & Tyler (1972a).

Cultured cells of *P.* T_3 are smaller than cultured cells of *P. trabecula* var. *mediolaeve*.

(i) Chemotaxis and sexual reproduction.

All clones isolated are heterothallic (Table 8). Clones UGL 2, 5, 13 and LS are maintained in pure culture and will form zygospores on desmid agar. The clones are plated alternately plus and minus, each occupying a quadrant. A few weeks after transfer when the edges of the clones are about 0.5 - 1 cm apart, plus clone cells directly adjacent to the minus clone suddenly become very active



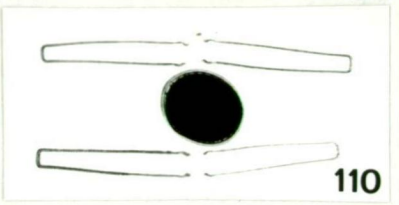


Fig. 108. Plus (on left) and minus clones on agar. Plus cells in the vicinity of the minus clone move actively towards it (arrows).

Fig. 109. Zygospore formation at the junction of plus and minus clones. x 25.

Fig. 110. Zygospore. x 125.

and move towards it (Fig. 108). The rest of the plus clone remains inactive. However, 1 - 2 weeks later all four clones become very active and quickly spread out. Pairing and zygospore formation occur at the junction of plus and minus clones (Figs. 109, 110).

Pairing, conjugation, zygospore characteristics and germination are similar to that of other *Pleurotaenium* species. The operculum is punctate.

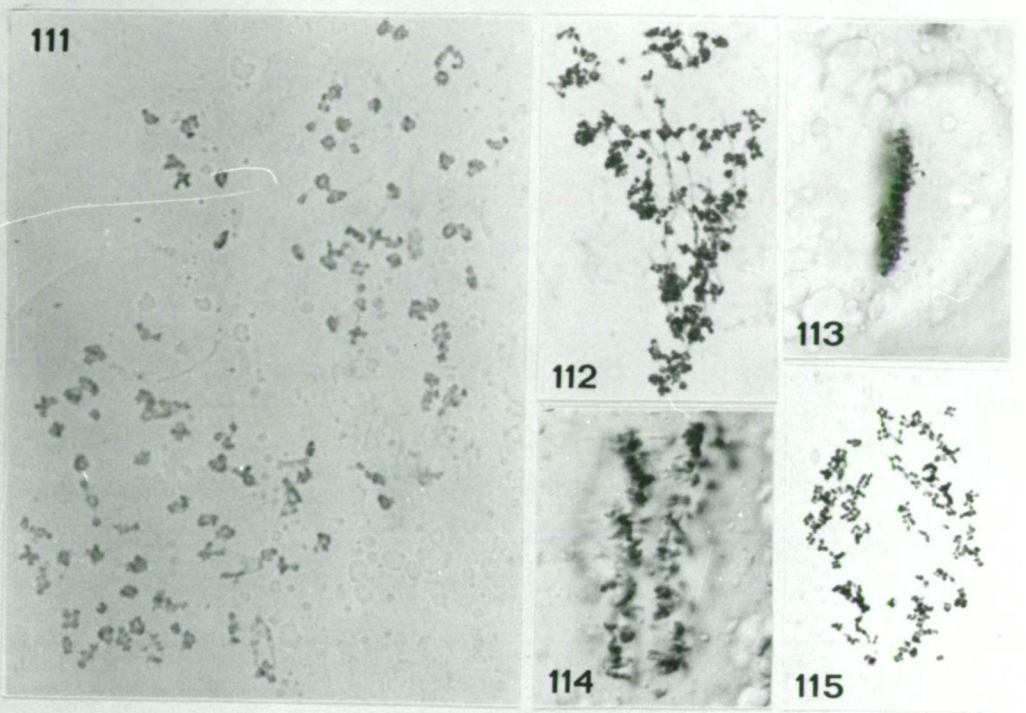
Attempts to cross *Pluerotaenium* T_3 with *P. trabecula* var. *mediolaeve* were unsuccessful.

(ii) Meiosis.

Only zygospores from YY 2 x WK were tested for germination. In common with the rest of the *Pleurotaenium* species, meiosis in *P.* T_3 is obscured by stickiness. A diakinesis preparation (Fig. 111) had much less stickiness than usual and about 100 bivalents could be counted. In most preparations the chromosomes are so badly stuck together that it is impossible to estimate the number of chromosomes present (Fig. 112).

Metaphase I to II follows the general pattern of *Pleurotaenium* meiosis. A metaphase I plate is formed (Fig. 113). Anaphase I is short-lived and probably synchronous (Fig. 114). There is stickiness at metaphase II and to further confound attempts at estimating the number of pairs of chromosomes, each chromatid has a clear or constricted middle giving it the appearance of a pair of dot-like chromosomes (Fig. 115).

A series of anaphase II to telophase stages, constructed from



Figs. 111 - 115. Meiosis in *Pleurotaenium* T_3.

Fig. 111. Diakinesis. x 750.

Fig. 112. Metaphase I with much stickiness, accentuated by squashing. x 600.

Fig. 113. M I plate, equatorial view. x 600.

Fig. 114. Anaphase I. x 1000.

Fig. 115. Metaphase II. x 700.

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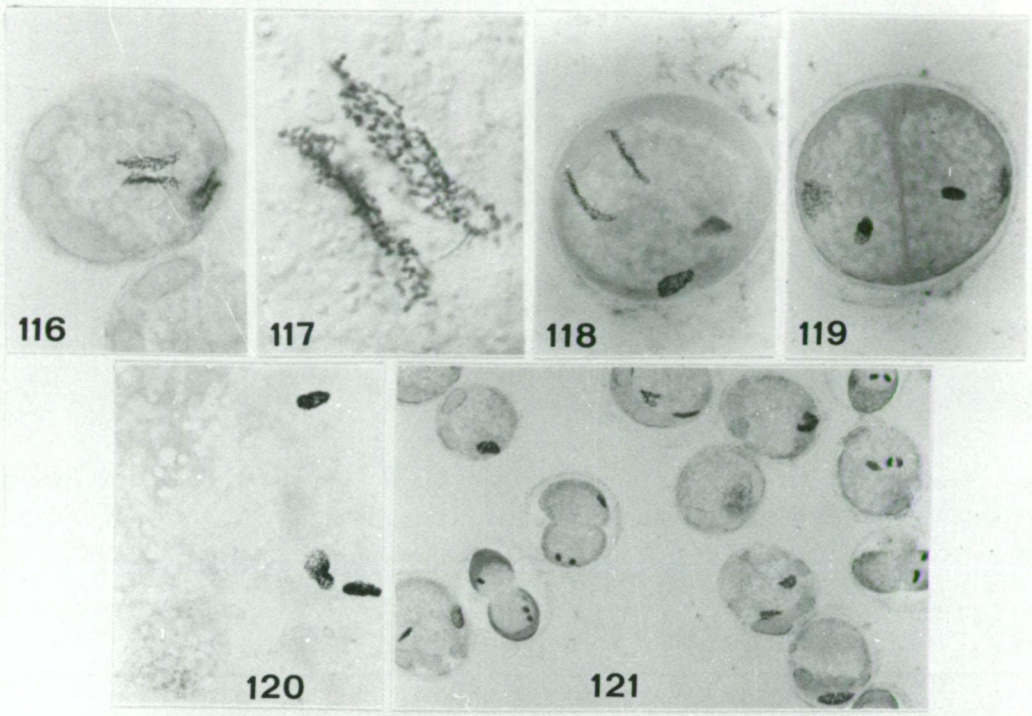

Figs. 116 - 121. Meiosis in *Pleurotaenium* T_3.

Fig. 116. Anaphase II. Both anaphases occurring more or less simultaneously. x 250.

Fig. 117. Enlargement of one of the anaphases II in Fig. 116. x 1000.

Fig. 118. Telophase. Two of the nuclei have turned pycnotic. x 250.

Fig. 119. Telophase. Beginning of cytoplasmic constriction. x 250.

Fig. 120. Three pycnotic and one surviving, fuzzy nuclei. x 250.

Fig. 121. Various meiotic stages. x 120.

several vesicles (Figs. 116 - 120) suggests that the two daughter nuclei from whichever is earlier of the two anaphases abort first, becoming pycnotic. One of the daughter nuclei of the later anaphase II survives to become the nucleus of the single gone.

Fig. 121 shows various stages of meiosis. The developing gones usually show only the three pycnotic nuclei as the surviving nucleus stains up lightly at best.

The F_1s are interfertile, forming zygospores which were germinated successfully.

A presumed diploid clone was recently isolated from YY 4.

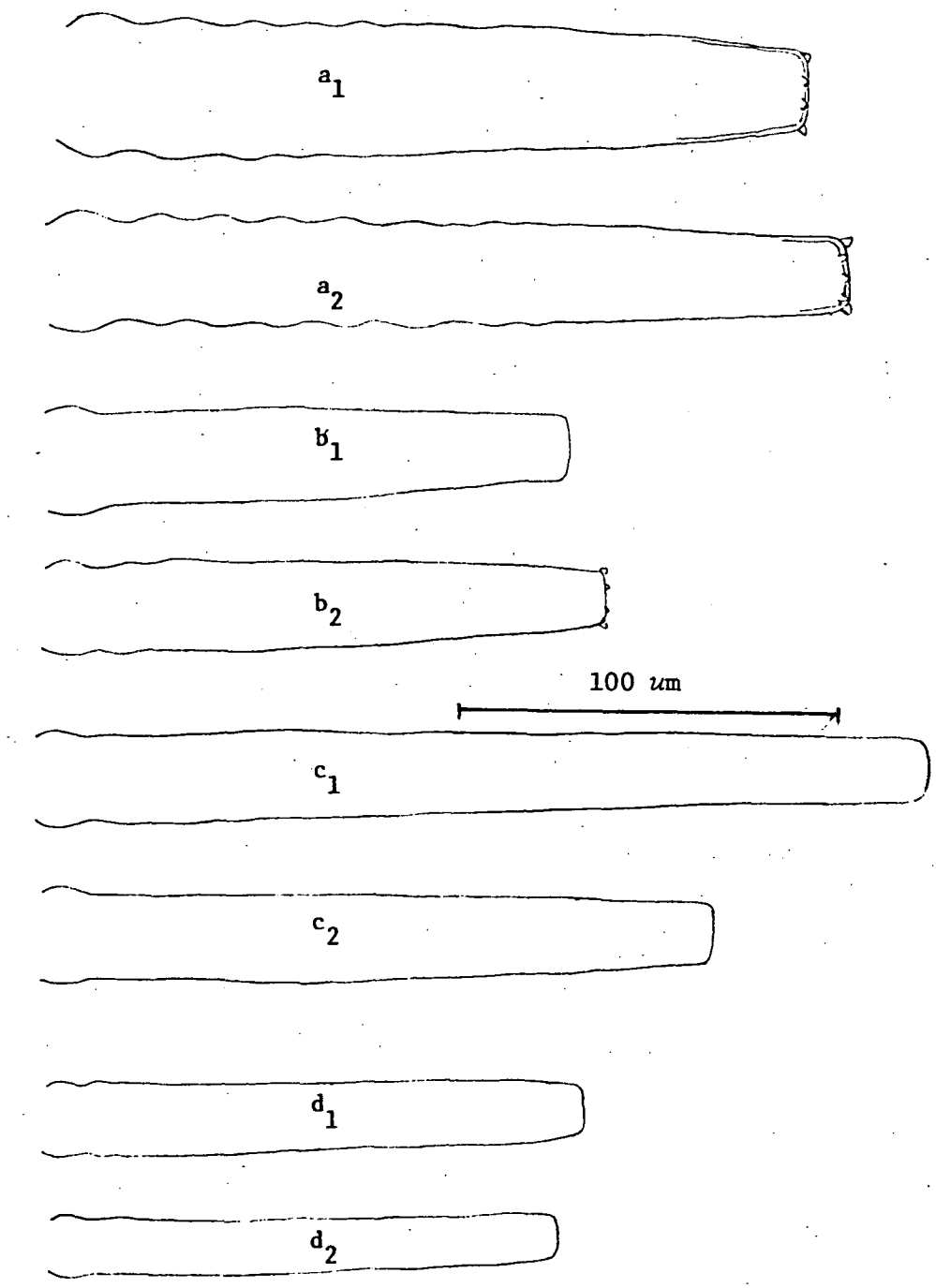

Fig. 122. Cultured cells of *Pleurotaenium* WK 3 (a_1, a_2), *P.* T_3 (b_1, b_2), *P.* T_4 (diploid, c_1, c_2; haploid, d_1, d_2).

8. Pairing between *Pleurotaenium* T_4, *Pleurotaenium* T_3 and *Pleurotaenium* WK 3.

Pleurotaenium WK 3 was originally isolated as one of three *P. coronatum* clones from Wartook Reservoir, Victoria. Other than their smaller size cultured cells of WK 3 are indistinguishable from those of *P. coronatum*. Dimensions of WK 3 cells are: 293-(364)-409 x 33-(36)-37 x 26-(28)-29 x 18-(19)-21 um. However, attempts to cross WK 3 with clones of both the *P. mamillatum* complex and *P. coroniferum* were unsuccessful.

WK 3 and clones of *Pleurotaenium* T_3 and *Pleurotaenium* T_4 were mixed in various combinations. So far only two crosses have produced "positive" results. In WK 3 x T_3 YY 2 usually one to two YY 2 cells would congregate round one WK 3 cell. Conjugation has not been observed. In T_3 UGL 13 x T_4 F_1 2n 1 some form of loose pairing was observed.

Fig. 122 compares the various cell types.

M. laticeps

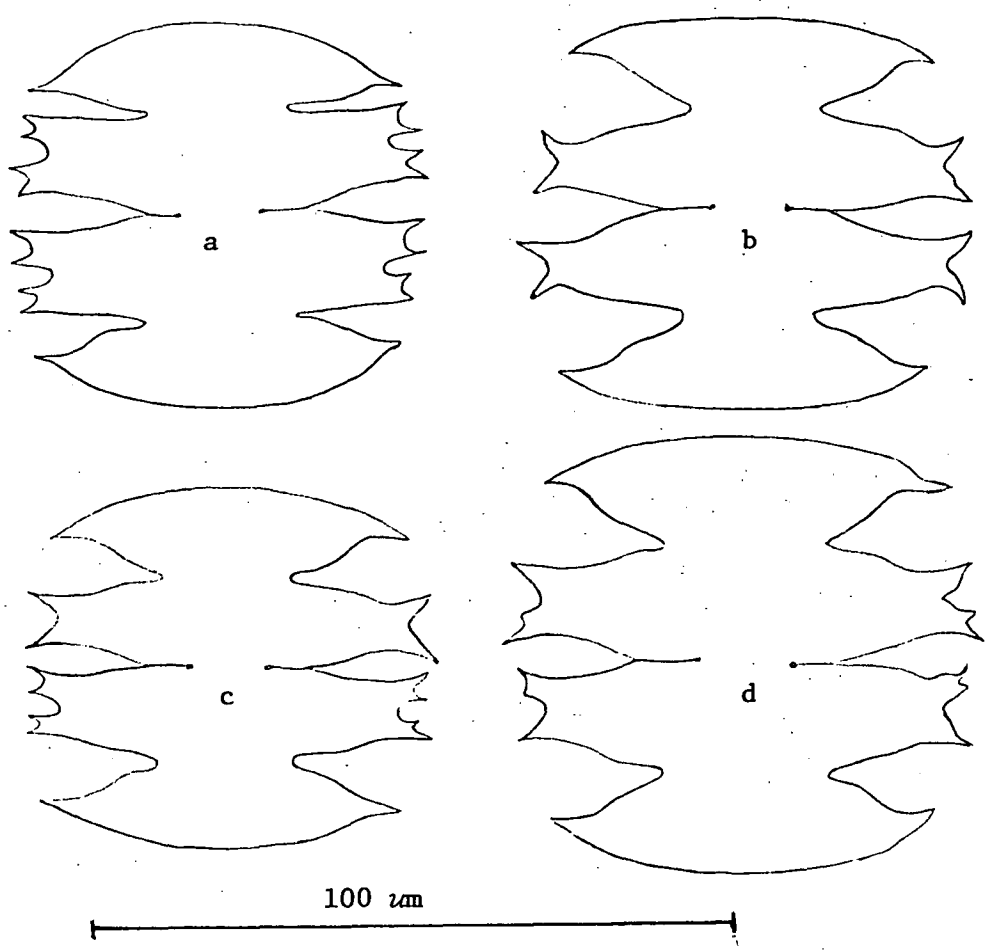

Fig. 123. a, Centennial Park; b - d, Upper Gunbower Lagoon.

Table 9. Source and mating type of *M. laticeps* clones.

Source	Mating type	
	+	-
Upper Gunbower Lagoon, Victoria.	UGL 1	UGL 2
	UGL 4	UGL 3
		UGL 5
Centennial Park, Sydney, N.S.W.	CP 1	CP 2
	CP 3	
	CP 4	
Tocumwal, N.S.W.	T 2	T 1

B. Sexual Reproduction, Polymorphism And Polyploidy In *Micrasterias*.

1. *Micrasterias laticeps* Nordst.

Taxonomy.

The cells are identifiable as *Micrasterias laticeps* Nordst. (Tyler, 1970; Bicudo & Sormus, 1972). Only cells with a large number of incisions in the lateral lobes (Fig. 123a) were observed from Centennial Park, Sydney, and Tocumwal and these maintain their wild morphology in culture. Upper Gunbower cells (Fig. 123b - d) are more heterogeneous, varying from cells with scarcely incised lateral lobes to cells like those from Centennial Park. In culture all cells tend towards the more incised type.

The cell wall is finely punctate and in a living cell fibrillar mucilage protrudes through the punctae.

Conjugation.

All clones isolated are heterothallic. The clones and their mating types are listed in Table 9. Both plus and minus mating types are found in each of the three localities.

The cells pair for conjugation and secrete an enveloping mucilage coat. Numerous granules of unknown origin dot this envelope (Fig. 124). The isthmuses lengthen, the semicells remaining joined by a thin new wall. From each cell a papilla protrudes towards the other conjugant. The papillae enlarge and eventually meet. Gamete fusion follows. Apparently, a thin wall is secreted by the papillae and is clearly visible as a puffed out tube as the young zygote

M. laticeps

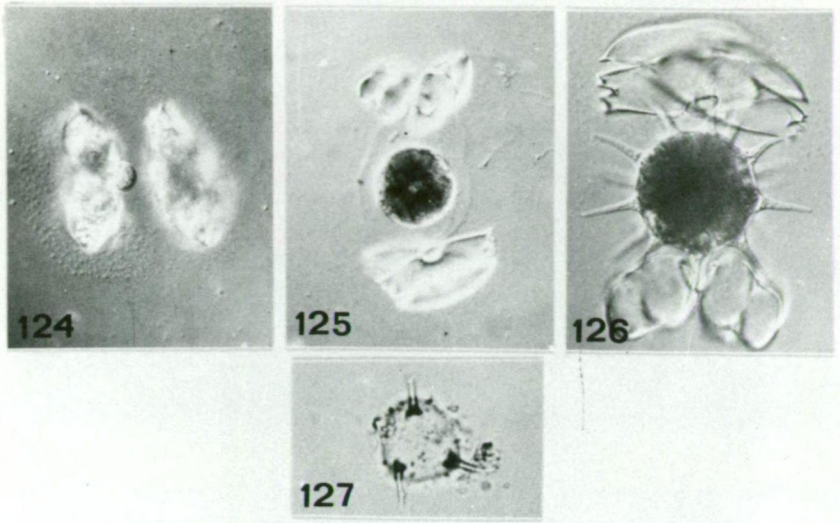

Fig. 124. Conjugants enclosed in granular mucilage. Cell on left has produced a papilla. x 200.

Fig. 125. Young zygote enclosed in thin, puffed out tube. x 200.

Fig. 126. Fully formed zygote, but still at green stage. x 300.

Fig. 127. An operculum bearing three spines. x 500.

condenses and assumes a spherical shape (Fig. 125). Small corrugations then begin to appear on the periphery, gradually increasing in size to become spines ornamenting the zygote (Fig. 126).

Conjugations begin around the 4th hour of the dark period and are usually over by the beginning of the light period. The first zygotes are formed from 3 - 8 days after mixing clones. In the maturation process the endopsore, mesospore and exospore are laid down. Part of the exospore forms an operculum, a simple disc which may bear up to three spines (Fig. 127).

Each pair of empty semicells of the conjugants can be seen to be joined by a cylinder of thin wall material and sometimes, especially in air-dried cells, a gamete exit pore is clearly visible.

At maturity the zygospore is globose and ornamented with strong, elongate and sometimes slightly bent spines. These spines are pointed, occasionally minutely hooked or bifid. Some zygospores have reduced spines. Two green chromatophores are just visible through the brown mesospore.

Zygospore germination.

Two month old zygospores may germinate about six days after immersion in fresh medium. Germination is usually erratic. For example, in one lot, four of the zygospores germinated six days after immersion. When ten days later no more zygospores had germinated the sample was dried and then rewetted, without any success. The third time the zygospores were dried and rewetted nothing happened until 16 days later when most of the zygospores germinated.

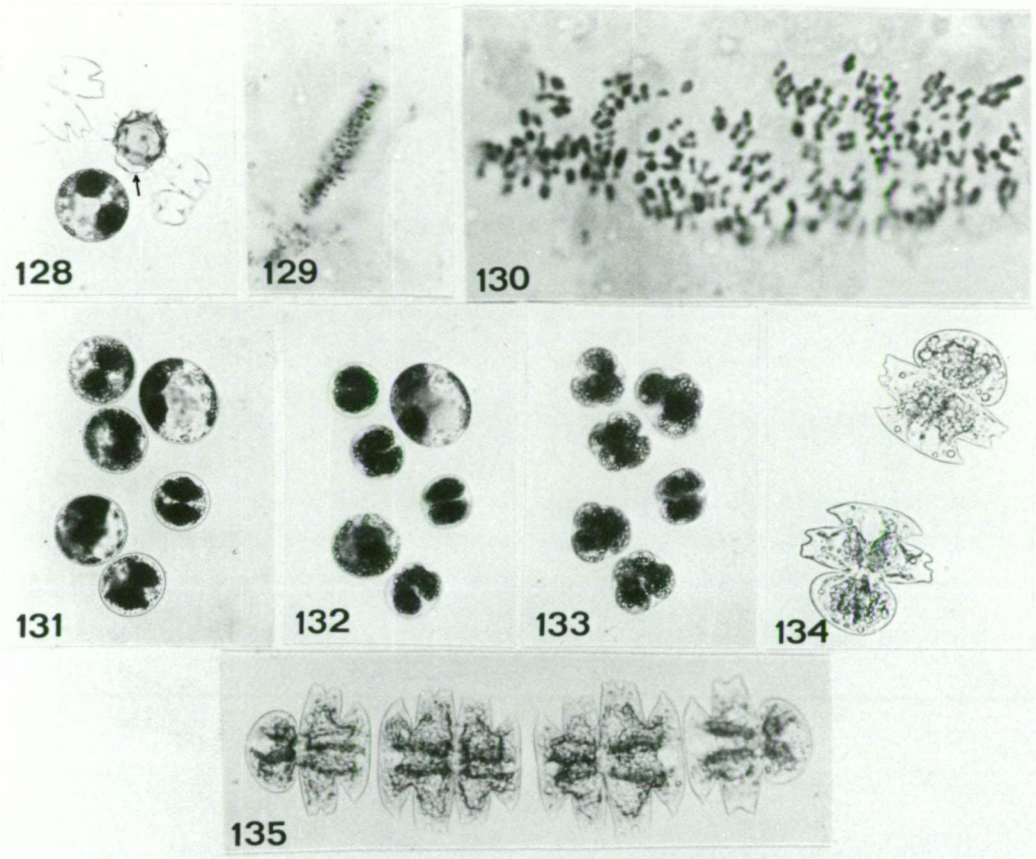

Fig. 128. Zygospore germination. Released vesicle with two chromatophores. Operculum (arrow). x 125.

Fig. 129. Metaphase I, equatorial view. x 700.

Fig. 130. Metaphase I. x 2000.

Figs. 131 - 133. Stages in the development of the germination vesicle.

Fig. 131. 1700 hours. About 1 hour after the vesicles were released. Cytoplasmic condensation. x 125.

Fig. 132. 2100 hours. First cytoplasmic cleavage. x 125.

Fig. 133. 0930 hours, following day. Vesicles each with two gones. x 125.

Fig. 134. First gonal division. New semicells assume characteristic shape of species. x 250.

Fig. 135. Second gonal division. Normal cells are formed. x 250.

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Zygospores from some crosses appear to germinate better than others.

In the process of germination the protoplast squeezes out past the operculum. A thin endospore encloses the freed protoplast. This germination vesicle is light grey in colour. Starch and oil globules occupy the periphery of a large, clear central vacuole. Two chromatophores are usually visible (Fig. 128).

The nucleus in a newly released vesicle is usually at metaphase I (Figs. 129, 130). The chromosomes are numerous and tiny, about half the size of *Pleurotaenium* chromosomes. Only two of the four meiotic products survive.

Gradually the protoplast shrinks, presumably by loss of water (Fig. 131). Cytoplasmic cleavage follows, dividing the protoplast into two halves (Fig. 132). The development of a furrow around the middle of each half results in the formation of the typical desmid form (Fig. 133). The two gones remain enclosed by the endospore which is eventually ruptured or hydrolysed. The gones do not have the shape characteristic of the species, but the new semicells formed at the first (Fig. 134) and subsequent divisions (Fig. 135) resemble the parental type.

The two products of a single zygospore are normally of opposite mating type.

Anomalous products.

One zygospore produced only a single large gone which divided in an unusual manner (Figs. 136 - 138) strongly suggesting the binucleate nature of the single gone. Two clones were isolated from the gone. These are of the same mating type.

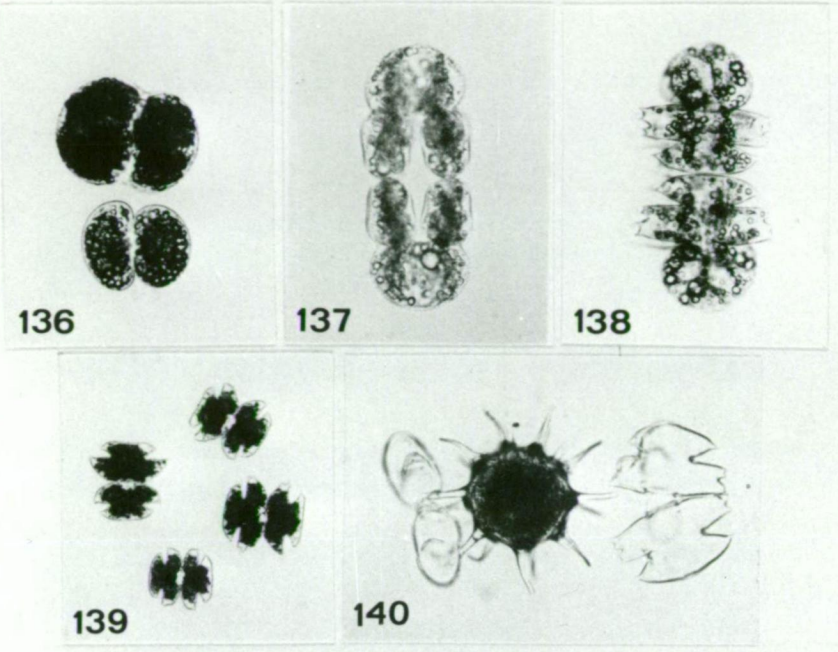

Fig. 136. Large gonal product. Normal product, at bottom, is included for comparison. x 250.

Fig. 137. Division of large gonal product. x 250.

Fig. 138. Broad side of Fig. 137. x 250.

Fig. 139. "Swing-wing" cells. x 125.

Fig. 140. Zygospore formed by conjugation between biradiate and small tri-radiate cells. x 250.

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Another product consisted of a round portion with two knobs protruding from it. This behaved like a normal binucleate cell, an isthmus and presumably a nucleus being present at each junction of a knob with the main portion.

An unusual cell was isolated from the F_1. The lateral lobes of each semicell sweep upwards towards the apex. The cell formed a small clone (Fig. 139) maintaining this new swing-wing cell shape. Growth was poor and the clone slowly died out.

(i). Inheritance of the 3-radiate character.

Small 3-radiate.

In two biradiate F_1 clones 3-radiate cells arose spontaneously. Dimensions of the two cell types are:

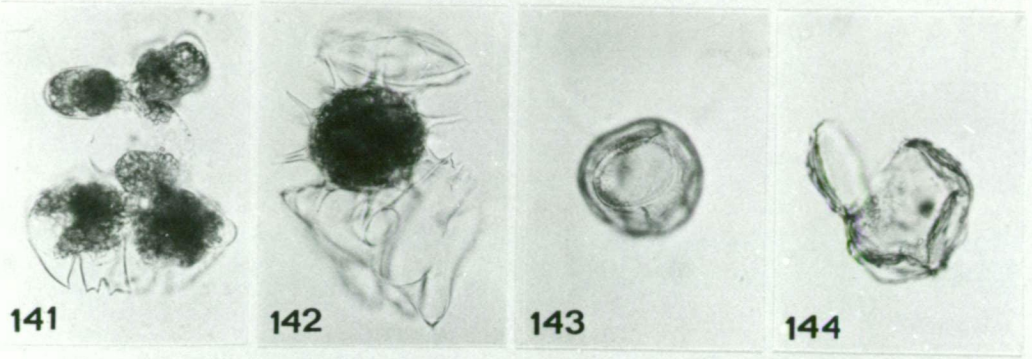
Biradiate 78-(82)-87 x 86-(90)-99 x 14-(15)-17 *um*.

3-radiate 59-(64)-68 x 65-(70)-77 x 15-(16)-18 *um*.

The 3-radiate cells appear smaller but are probably comparable to the biradiate in volume because of the extra radiation.

Isolated 3-radiate clones soon contain biradiate cells indicating a slow reversion to the biradiate character. Preliminary observations suggest that this reversion is permanent and is much more rapid when 3-radiates are grown with biradiates than when the 3-radiate cultures are kept pure.

The 3-radiates will form viable zygospores with biradiates (Fig. 140). So far no 3-radiates have been recovered from the progeny of several such crosses suggesting that the 3-radiate trait is not directly under genetic control. The ease of reversion of



Figs. 141 - 144. Haploid biradiate x large 3-radiate.

Fig. 141. Conjugation. x 250.

Fig. 142. Semi-mature zygospore. x 250.

Fig. 143. Empty, unadorned zygospore coat showing operculum. x 250.

Fig. 144. Spore coat with operculum hinged outwards. x 250.

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3-radiates to biradiates would tend to support this.

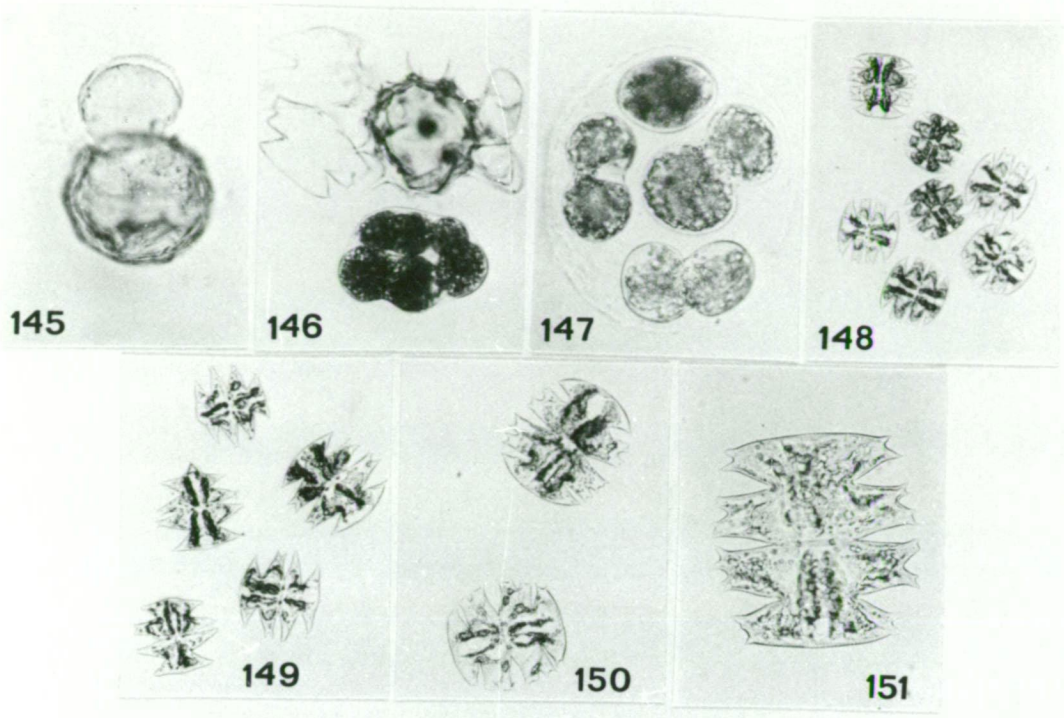
As both clones are of the same mating type the behaviour of the progeny from a 3-radiate by 3-radiate cross is unknown.

Large 3-radiate.

In clone UGL 1 several cells which were larger than normal were observed at the edge of the clone. When isolated these cells multiplied to form a culture (UGL 1 2n) in which all the cells were large, no reversions to the normal smaller type have ever been found. When first isolated the large cells were biradiate, as were the normal cells, but the resulting culture soon began to contain a number of 3-radiates. These eventually became the dominant form. Rarely, 4-radiate cells are formed. Dimensions of large 3-radiate cells are: 98-(103)-111 x 87-(91)-95 x 20-(22)-24 μm.

The large cells have the same mating type as their normal "parents" producing zygospores with compatible clones (Figs. 141, 142). The only significant difference between large x normal and normal x normal is the tendency towards spine reduction in zygospores in the former crosses.

Zygospore germination is normal. As most of the zygospores lack or have reduced spines, the opercula are more easily observed. The operculum is identical to the exospore in structure. On germination it is hinged outwards and is rarely detached completely (Figs. 143, 144). In empty zygospores the mesospores do not show any tell-tale jagged signs of rupture and careful examination revealed that each mesospore too bears a sort of operculum corresponding to the



Figs. 145 - 151. Products from haploid biradiate x large 3-radiate.

Fig. 145. Exospore operculum with brown mesospore operculum appressed to it. x 250.

Fig. 146. Germination vesicle with three gones. x 200.

Fig. 147. Germination vesicle with four gones. x 350.

Fig. 148. Large F_1s. x 100.

Fig. 149. F_1s with narrow tapering lobes. x 125.

Fig. 150. F_1s with rounded outlines. x 160.

Fig. 151. Cell with bifid apical lobes. x 300.

exospore operculum in size and position and appears to be closely appressed to it (Fig. 145).

Two gones, slightly larger than normal may be produced by each zygospore, but many of the released vesicles do not develop normally. The gones are often deformed or one of the gones may be normal while the other is a binucleate. Several vesicles each containing 3 gones (Fig. 146) and two vesicles each containing 4 gones (Fig. 147) were isolated and the gones separated. One surviving clone or none per vesicle was the usual result. Only once were two clones raised from a 3-gone vesicle. Only a small percentage of the F_1 are viable.

In another batch of zygospores, germination proceeded to the gonal stage, a few reached the first gonal division, but no further development took place even though the products remained green. When the medium was replaced with a different soil-water, the products divided actively.

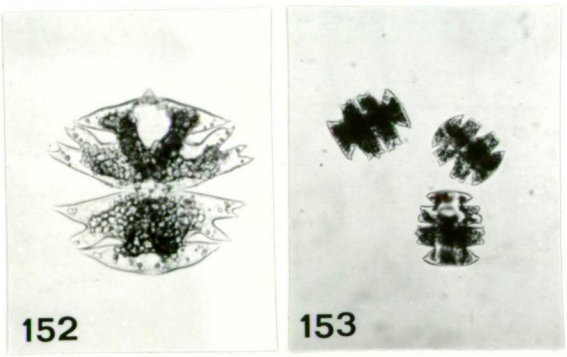
Several weeks later it was obvious that the mixed culture contained cells in a wide variety of shapes and sizes. The size differences range from 2/3 to twice normal size (Fig. 148). The largest of these cells tended to have more incised lateral lobes.

Of cells with shapes significantly different from normal, skinny-looking cells (Fig. 149) are the most common. Even in these there is a variety of sizes and a gradation in shape towards the normal. Figures 150 and 151 show three new cell types. The apical lobes of the cell in Fig. 151 have bifid ends reminiscent of *Micrasterias pinnatifida.*

The most interesting is certainly the bizarre cell depicted in

Fig. 152. The cell died after isolation. A few 3-radiate cells were also observed (Fig. 153). In addition to these new cell types, cells which can only be regarded as abnormalities or monstrosities were also common.

Three other presumed diploid clones were recently isolated from a mixed F_1 culture of a CP 3 x UGL 5 cross. Unfortunately all three clones are of the same mating type as UGL 1 2n.



Figs. 152 - 153. Haploid biradiate x large 3-radiate, F_1s.

Fig. 152. Bizarre cell. x 250.

Fig. 153. 3-radiate cells. x 100.

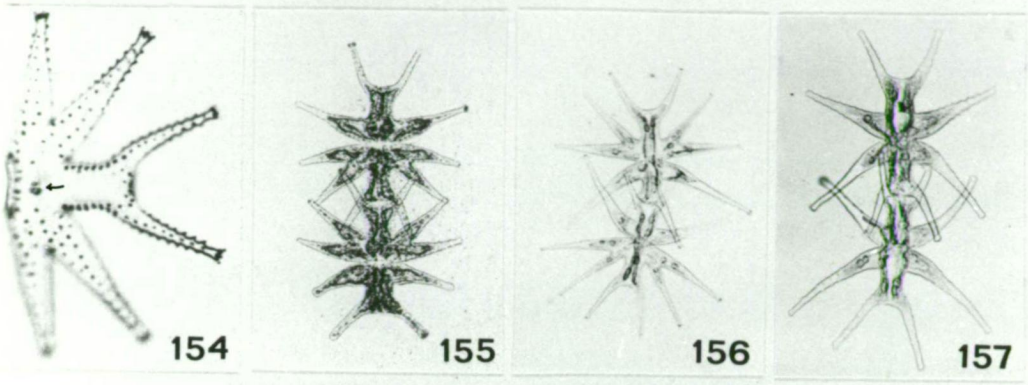


Fig. 154. Air dried empty semicell, ex-culture. Arrow points to central tumour. x 300.

Fig. 155. *M. berganii*-like cell producing *hardyi* new semicells. x 125.

Figs. 156 - 157. Production of *M. berganii*-like cells in a special liquid medium.

Fig. 156. Production of *berganii*-like semicells. x 125.

Fig. 157. Division of *berganii*-like cell. x 150.

33/3/LL

2. *Micrasterias hardyi* G. S. West.

Taxonomy.

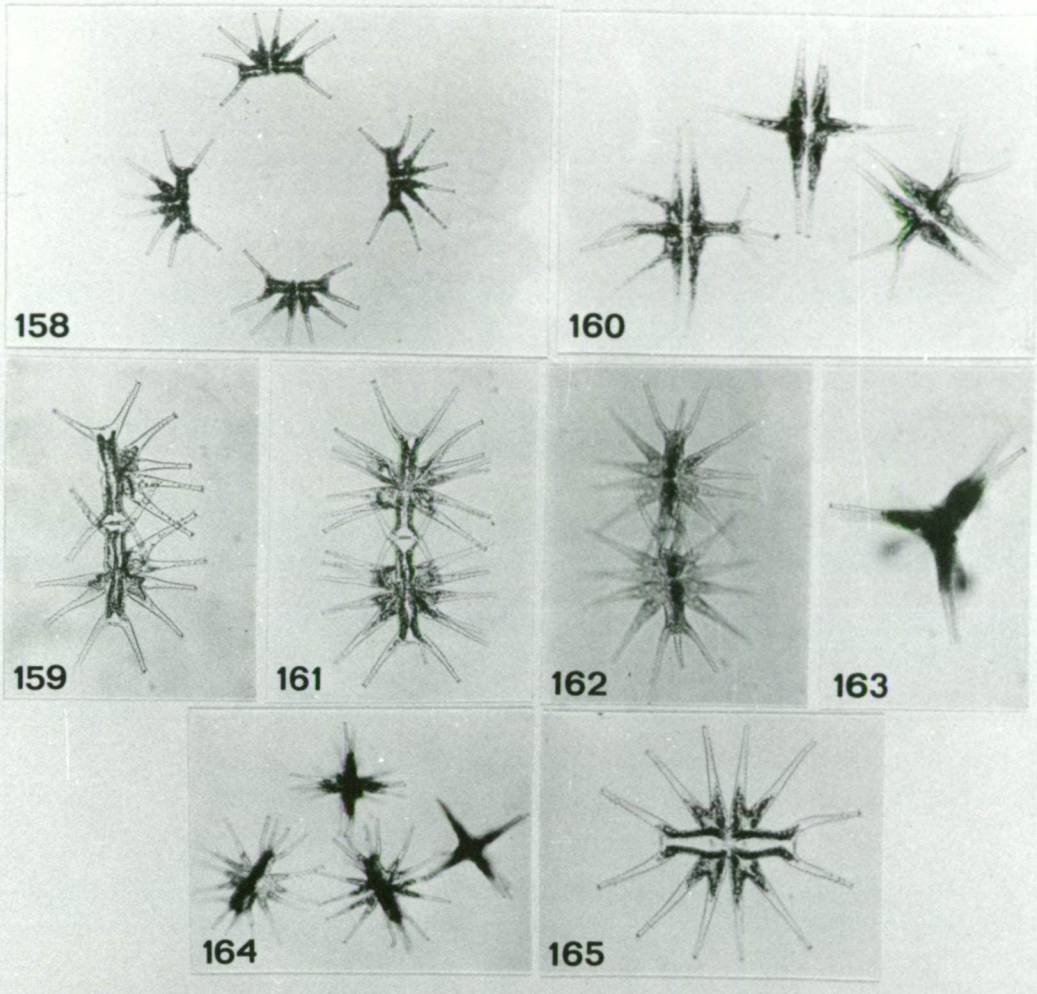
The plants agree with descriptions and figures of *Micrasterias hardyi* G. S. West (West, 1905, 1909; Hardy, 1906; Tyler, 1970). An additional feature not mentioned in previous descriptions is the presence of a central tumour (Fig. 154). Large pores punctate the end wall.

Clones WL 1, WL 2 were isolated from Woods Lake, Tasmania; W 1 from Wartook Reservoir, Victoria; and M 1, M 2 from Lake Mulwala, New South Wales.

Observations.

In culture the new semicells formed may, rarely, assume a *Micrasterias berganii* Hauge characteristic. Occasionally complete *berganii*-like cells were observed. Observation of single isolates through several divisions revealed that the character is unstable, the cells reverting to the *M. hardyi* shape usually in the first division (Fig. 155).

In an attempt to find an optimum soil-water medium for this species, cells were transferred into several soil-water media. In one of the media all the new semicells formed during the first few divisions assumed the *berganii* shape (Figs. 156, 157). The medium, however, was unsuitable as no further divisions took place, the cells eventually dying. Subsequent attempts at duplicating this strange behaviour were unsuccessful.



Figs. 158 - 165. Morphological variants and derivative clones of WL 1.

Fig. 158. Four cells each with four arms missing. x 80.

Fig. 159. Janus cell producing identical new semicells each of which lacks two arms. x 125.

Fig. 160. Cells of clone MV II. Cell on right is typical of cells of the clone. x 125.

Figs. 161 - 165. Cells of WL 1 2n.

Fig. 161. Division of large cell with several extra arms. x 100.

Fig. 162. Division of large 3-radiate cell. x 100.

Fig. 163. End view of large 3-radiate cell. x 125.

Fig. 164. Large 4-radiate cells. x 75.

Fig. 165. Large biradiate cell. x 125.

Clone WL 1 has in addition produced the following three types of cells:

(i) Morphological variant I. From an old culture four variant cells (Fig. 158) were isolated. The cells see-sawed between producing normal and variant semicells (Fig. 159) for several divisions before reverting to normal.

(ii) Morphological variant II. These cells (Fig. 160) commonly lack all the median arms and the lateral arms on both semicells tend to run parallel instead of diverging. Sometimes a single straight arm replaces the two apical arms. The chloroplasts are usually disorganised. The variation is stable, no reversions to normal have ever been observed. Clone MV II was established from one of the cells.

(iii) Large cells, clone WL 1 2n. Several cells with extra arms (Fig. 161) were isolated. Growth was originally poor but a healthy culture maintaining the large size was established.

After several weeks two types of 3-radiate cells began to appear in the culture. One type has an angle of about 120° between one radiation and the next (Figs. 162, 163) while the other rare type has two of the radiations horizontal with the third perpendicular to this.

The present culture contains biradiate, 3-radiate, 4-radiate (Fig. 164), janus 2+3, 2+4 and 3+4 cells; each type with or without extra arms. The proportions of the various cells types are listed in Table 10. Except for size the biradiate cells without extra arms (Fig. 165) are superficially identical with normal cells.

Table 10. Types of cells and their relative numbers in WL 1 2n of *M. hardyi*.

Biradiate		J 2+3	3-rad.	J 2+4	4-rad.	J 3+4	Half *alata*
No. of extra arms							
0	101	3	42	1	2	1	3
1	34						
2	21						
3	8						
4	6						
5	2						
6	1						
8	2						
Total	175	3	42	1	2	1	3

Table 11. Dimensions of cultured strains of *M. hardyi* (um).

	Length	Breadth	Isthmus
Haploid biradiate	188-(195)-203	143-(163)-185	16-(18)-20
Haploid 3-radiate	128-(148)-161	95-(116)-131	18-(20)-23
Diploid biradiate	239-(261)-278	212-(235)-263	26-(28)-29
Diploid 3-radiate	224-(236)-254	164-(191)-227	26-(29)-31

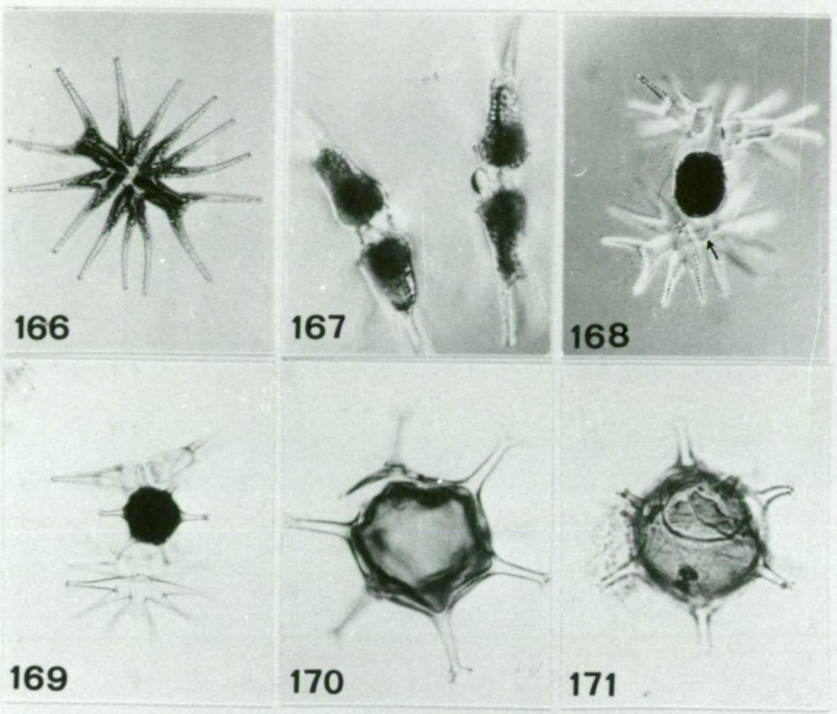


Fig. 166. Large cell with one semicell the shape of a semicell of *M. alata*. x 125.

Figs. 167 - 169. Conjugation in *M. hardyi*.

Fig. 167. Production of conjugation papillae. x 250.

Fig. 168. Immature zygote. Conjugation tube visible near lower cell. Arrow points to gamete exit pore. x 125.

Fig. 169. Mature zygospore. x 125

Fig. 170. Spore coat with operculum extended slightly outwards. x 300.

Fig. 171. Spore coat with discoid operculum and ruptured brown mesospore. x 300.

Dimensions of the various cell types are given in Table 11. The difference between large and normal cells is significant.

An interesting abnormality was observed in several semicells of WL 1 2n. Each of these semicells (Fig. 166) has triple lateral processes reminiscent of *Micrasterias alata* Wall. (Tyler, 1970; Fig. 18A, 18B).

Conjugation.

All clones isolated are heterothallic. Clones WL 1 and its derivatives are plus in mating type whereas WL 2, M 1 and M 2 are minus.

The conjugation process is similar to that of *M. laticeps*. The cells pair and secrete a mucilage envelope. Unlike that of *M. laticeps* this is agranular. The isthmuses lengthen as a thin new cylindrical wall is formed. Conjugation papillae are produced (Fig. 167). These touch and gamete fusion occurs. As in *M. laticeps* a puffed out tube is visible round the young spherical zygote (Fig. 168).

Zygospores are formed about a week after mixing clones. Most crosses produce few zygospores. Though many more conjugations occurred these were unsuccessful, the gametes lysing.

The mature zygospore is globose and ornamented with grapnel-like processes (Fig. 169). A circular disc of the exospore forms an operculum (Figs. 170, 171). Two green chromatophores are sometimes visible through the brown mesospore.

The morphological variant II and large cell strains are similar

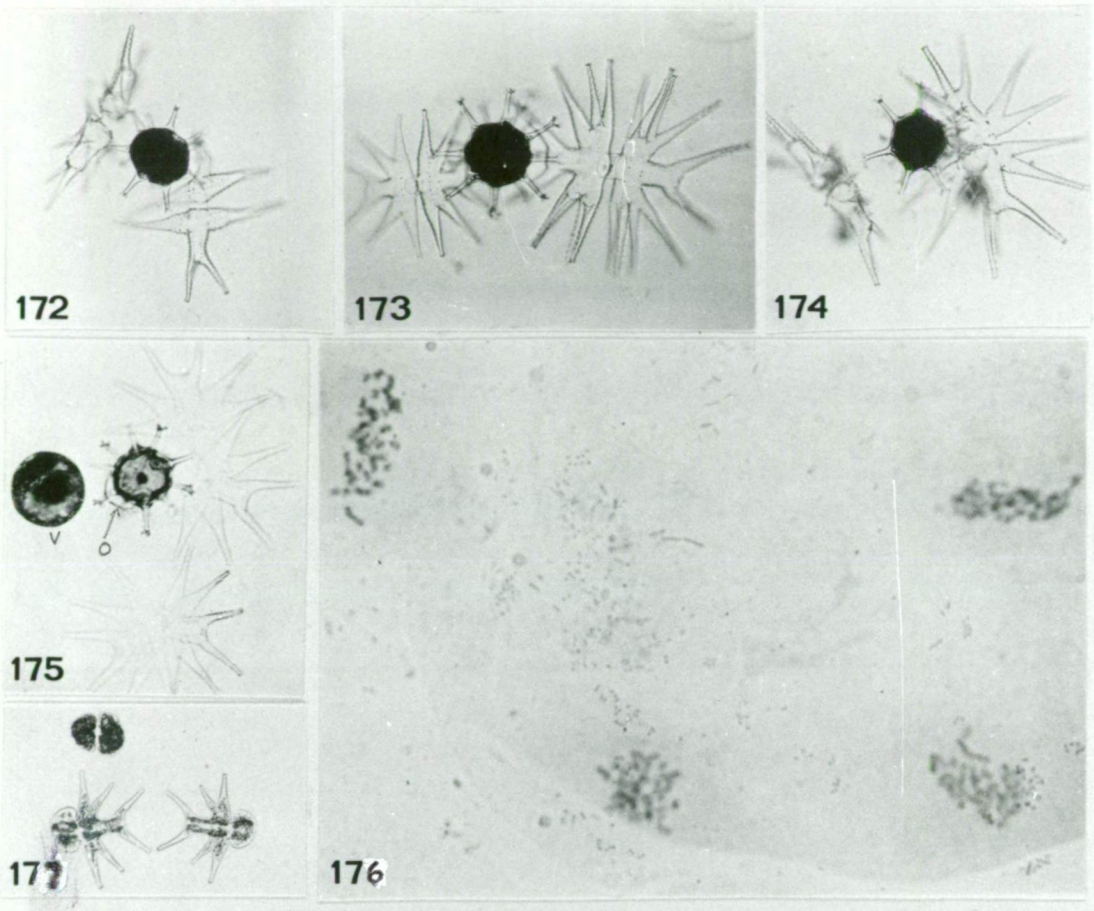


Fig. 172. M 1 x MV II zygospore. Variant cell (MV II) on lower right. x 125.

Fig. 173. Zygospore from normal haploid by large cell. x 125.

Fig. 174. Zygospore from normal haploid by large 3-radiate. x 125.

Fig. 175. Zygospore germination. v = vesicle with two chromatophores, o = operculum. x 125.

Fig. 176. Meiosis. Early telophase. x 1260.

Fig. 177. Two gones, one recently divided. x 125.

to their normal parent strain in sexual activity, forming zygospores with compatible strains (Figs. 172 - 174). A mixture containing M 1 and the plus strains WL 1, MV II and WL 1 2n produced zygospores in each combination.

A recent sample from Toolondo Reservoir, Victoria, contained many *M. hardyi* cells. Zygospores were formed when the sample was subjected to conditions conducive to conjugation.

Zygospore germination.

The zygospores are difficult to germinate. One lot of zygospores from a cross between M 1 and WL 1, MV II and WL 1 2n was alternately dried and rewetted five times before any F_1 were isolated. A few zygospores germinated the third time, a little more the fourth and fifth times.

At the onset of germination the mesospore is ruptured as the protoplast, enclosed by the endospore, squeezes out past the operculum (Fig. 175). Two chromatophores are usually visible.

Very few squashes were made because of the scarcity of vesicles. An early telophase preparation (Fig. 176) indicates a small complement of between 20 - 30 chromosomes for *M. hardyi*. After meiosis the process of shrinking, cytoplasmic cleavage, development of the two gones and the division of the gones to produce *hardyi* semicells (Fig. 177) is similar to that in *M. laticeps*.

Only a few of the vesicles developed to the gonal stage. Most of the gones ruptured in the process of dividing. Sometimes *berganii*-like semicells were produced in the first few gonal

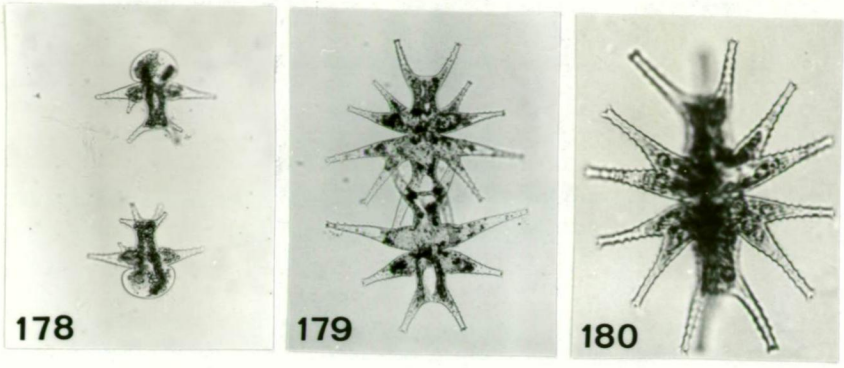


Fig. 178. Unusual semicells produced in the first gonal division. x 125.

Fig. 179. Janus *hardyi-berganii* cell producing dissimilar new semicells. x 150.

Fig. 180. Small 3-radiate cell. x 250.

divisions (Fig. 178). None of these *berganii* cells was stable and reversion to the *hardyi* shape is usually rapid, often in the second gonal division. Fig. 179 shows division in one of the janus cells. The two new semicells are quite different and each appears to assume the shape of its "parent" semicell.

A few M 1 x MV II and M 1 x WL 1 2n zygospore germinations were observed. None developed past the vesicle stage.

Attempts to germinate the Toolondo zygospores were unsuccessful.

3-radiate F_1 clone.

From a slightly larger than normal gone of unknown origin a 3-radiate clone was established. Cells of the clone (Fig. 180) appear to be smaller than the normal haploid biradiate (Table 11) but are most likely comparable in volume because of the extra radiation. There is, therefore, no suggestion of diploidy.

The 3-radiate character was maintained for several months, but recently a few biradiate cells were observed in the culture.

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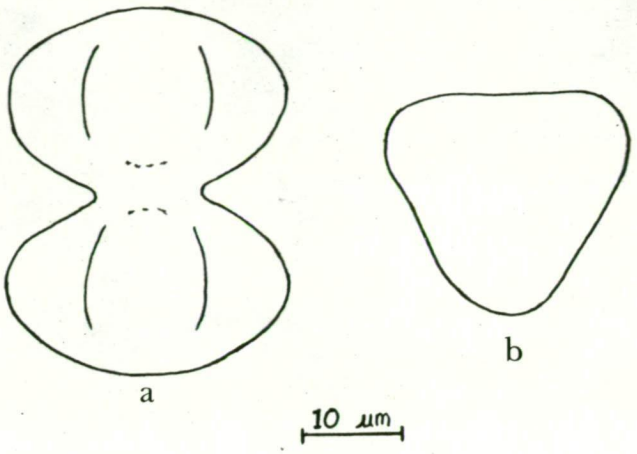


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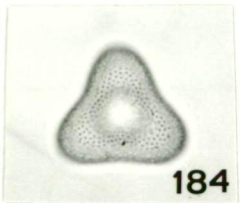
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Fig. 181 a, b. Side and end views respectively of wild cell.

Fig. 182. Side view of cultured cell. x 500.

Fig. 183. End view of cultured cell. x 500.

Fig. 184. End view of air-dried empty semicell, ex-culture. x 500.

C. Chloroplast To Germination Product Number Relationship In *Staurastrum orbiculare* var. *ralfsii* West & West.

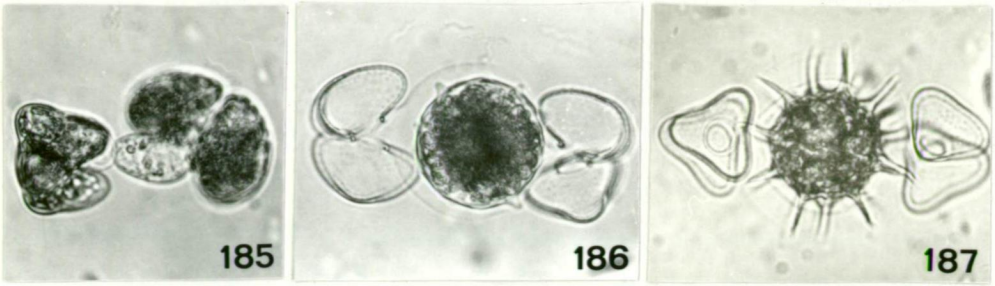
Taxonomy.

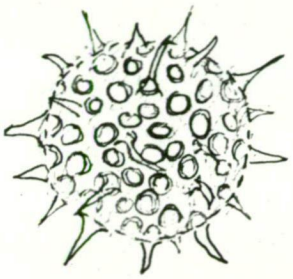
Wild cells are small, about 1.2 times as long as broad. Each semicell is transversely elliptic (Fig. 181a) and triangular in end view with concave sides and broadly rounded angles (Fig. 181b). Dimensions of 7 cells from Arthurs Lakes, Tasmania, are: length, 31-(33)-35; breadth, 24-(26.3)-30; isthmus, 7.5-(8.3)-9 *um*.

In general, basic morphological features of wild cells are retained in culture, but the semicells are transversely sub-triangular (Figs. 182, 183). As in wild cells the wall is smooth and finely punctate (Fig. 184). Dimensions of 10 cells from culture are: 24-(30.2)-34 x 24-(28)-32 x 10-(10.4)-11 *um*. Zygospores produced in culture (Fig. 187) are spherical and adorned with numerous, acute spines. Dimensions of 20 zygospores without spines are 29-(30.6)-33 *um*. The spines average 11.5 *um*.

Cells from the wild population agree with figures and descriptions of *St. muticum* Breb. (West & West, 1911, p. 133 & Pl. 118, Figs. 16 - 20), the cells usually being transversely elliptic. However, zygospores of *St. muticum* are globose and furnished with stout spines which are bifurcate at the apices (West & West, 1911).

From culture studies the cells are more closely identified with *St. orbiculare* var. *ralfsii*, "each semicell is subtriangular ..... zygospore globose, furnished with numerous, simple, acute spines" (West & West, 1911, p. 156 & Pl. 124, Figs. 12, 13, 15, 16).





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Figs. 185 - 187. Conjugation in *St. orbiculare* var. *ralfsii*

Fig. 185. Production of conjugation papillae. x 500.

Fig. 186. Young zygote with developing spines. The conjugation tube is visible around the zygote. x 500.

Fig. 187. Mature zygospore. x 500.

Fig. 188. Exospore ornamentation.

A culture of *St. orbiculare* Ralfs var. *ralfsii* was obtained from the Culture Collection of Algae and Protozoa, Cambridge. The cells are smaller than those of the Arthurs Lakes clones and will not cross with them. There is some doubt about the identity of the Cambridge strain. It came originally from the Prague Collection as a *Cosmarium*.

Sexual Reproduction.

Two clones were cultured from the two products of a zygospore produced in mixed culture. The clones are heterothallic and opposite in mating type.

The conjugation process is similar to that in *Micrasterias*. The cells pair and secrete a mucilage envelope. In each cell the semi-cells detach at one side as the conjugation papilla is produced (Fig. 185). The papillae eventually fuse, forming a tube where gametogamy occurs. Undulations on the periphery of the zygote gradually enlarge into acute spines (Figs. 186, 187).

The mature zygospore is more ornate than that of *Micrasterias*. Between the spines, on the exospore, are irregular rings of ridges. The wall inside the rings appears to be thinner, giving the zygospore an overall cratered appearance (Fig. 188). The mesospore is light brown and the endospore is thin and transparent. Two chromatophores can usually be seen through the light-brown spore coat. Zygospores such as this were observed in an unidentified species of *Staurodesmus* presently under culture. Teiling (1967) created the new genus *Staurodesmus* to include the smooth-walled monospinous species previously under *Staurastrum* and *Arthrodesmus*. *St. orbiculare*

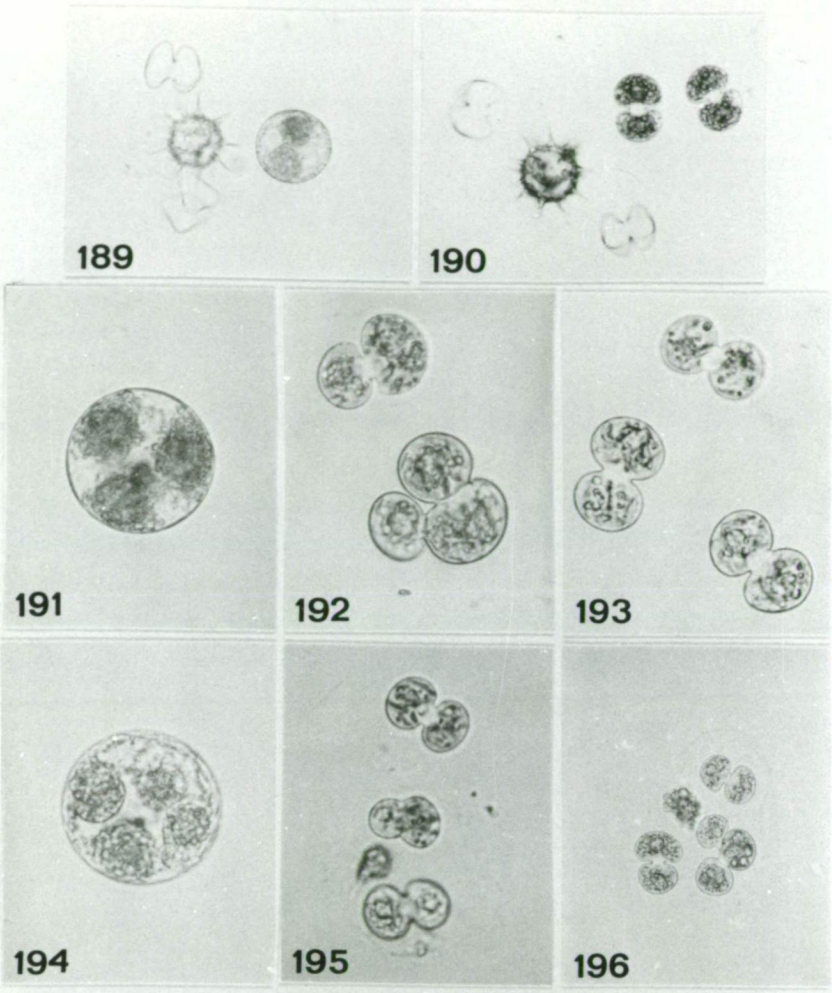

Fig. 189. Newly released germination vesicle with two chromatophores, x 250.

Fig. 190. The two products from a zygospore. x 250.

Fig. 191. Vesicle with three chromatophores. x 500.

Fig. 192. One normal and one Siamese twin products from a vesicle with three chromatophores. x 500.

Fig. 193. 3 products from a 3-chromatophore vesicle. x 500.

Fig. 194. Vesicle with 4 chromatophores. x 500.

Fig. 195. 3 products and a blob from a 4-chromatophore vesicle. x 400.

Fig. 196. 2 gones, 1 Siamese twin and a blob from a 4-chromatophore vesicle. x 250.

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var. *ralfsii* looks like a *Staurodesmus* except that it lacks spines.

After a short period of maturation, germination of the zygospores occursupon immersion in fresh medium. Both the mesospore and exospore are ruptured releasing the germination vesicle. There is no operculum. Like the *Micrasterias* vesicles, *St. orbiculare* var. *ralfsii* ones are light-grey in colour and lack prominent haematochrome granules. Each vesicle normally contains two chromatophores (Fig. 189). After meiosis two products are formed by cleavage and constriction of the protoplast (Fig. 190).

Rarely, vesicles with three or four chromatophores were observed (Fig. 191, 194). Eight and three vesicles each with three and four chromatophores respectively were placed on desmid agar and kept under observation. The results are presented in Table 12 Figs. 191 - 193 and Figs. 194 - 196 show results from some 3-chromatophore and 4-chromatophore vesicles.

From the results it is clear that vesicles with more than two chromatophores tend to produce more than the normal number of two products. If development were normal 3-chromatophore and 4-chromatophore vesicles can be expected to produce 3 and 4 products respectively.

Where there are more than two products, these are usually smaller and it is difficult to raise clones from them.

The simple mechanics of conjugation and zygospore germination in *St. orbiculare* var. *ralfsii* formed part of an Honours thesis submitted by the author to the University of Tasmania.

Table 12. Development of *St. orbiculare* var. *ralfsii* germination vesicles each with either 3 or 4 chromatophores.

Number of vesicles with 3 chromatophores	Eventual development of each vesicle
1	Died
2	2 products and dead blob
2	Monstrous product with 5 - 6 semicells
2	1 product and 1 Siamese twin
1	Three products
Number of vesicles with 4 chromatophores	
1	Died
1	Three products and dead blob
1	2 products, 1 Siamese twin and dead blob

DISCUSSION

Desmids rarely form zygospores in nature. Lund (Teiling, 1950) reported that a five year period of weekly examination of plankton from Lake Windermere failed to produce a single zygospore even though the lake is very rich in desmids, both in quantity and number of species. However, in small ponds or shallow pools desmid zygospores are more frequently found (Fritsch, 1953).

Starr (1959) cited unsuitable ecological conditions as limiting factors to zygospore formation in nature. Coesel and Teixeira (1974), obtaining only three sexual strains out of over a hundred under culture, argued that a lack of sexual potential provides the best explanation.

The majority of clones used in this investigation were isolated at random with no prior knowledge that they would be fertile. Most strains are heterothallic. In several cases both plus and minus mating types were isolated from the same locality yet zygospores of the particular species were previously unknown or not met with in spite of frequent plankton collections from the site. The sexual potential is certainly there. Perhaps the desmids are too dispersed, or more probably, suitable ecological conditions are lacking.

There is evidence to suggest that some lakes may be populated by only a single mating type, if not a clone, of the species. For example, all eight clones of *P. mamillatum* from Yan Yean are of the same mating type. It thus appears that a combination of heterothallism and unsuitable environmental conditions account for the rare occurrence

of zygospore formation in nature.

Both these factors may most frequently be overcome in ephemeral ponds and pools. Their very ephemeral nature favours colonization through resistant spores, thereby producing a potentially fertile population. Suitable conditions for conjugation occur with the drying up of the pond (Fritsch, 1953).

In the laboratory sexual reproduction in most desmids has been induced routinely only when cultures were exposed to an atmosphere rich in carbon dioxide or when subjected to nitrogen deficiency, and often under a different light and temperature regime (Starr, 1955a; Biebel, 1964; Ichimura, 1971). In contrast the *Pleurotaenium* species studied here are remarkable in that to induce conjugation it was necessary only to mix compatible clones under conditions no different to those for vegetative growth.

It has been suggested that zygospores are formed usually at the advent of or under adverse conditions and that the zygospores are a means of surmounting these unfavourable conditions (Fritsch, 1953). Another, and perhaps much more important reason for zygospore formation is that of exchange of genetic material in which case one would expect zygospore formation to occur whenever compatible cells come into contact under normal growing conditions, such as in *Pleurotaenium.*

What brings the cells together in the initial pairing process prior to actual conjugation is not well understood. Brandham (1967) observed an "anisogamous" pairing behaviour in *Cosmarium botrytis* where cells of one clone become relatively passive while those of

the other clone move actively towards them. He suggested that the behaviour is caused by the probable production of one or more hormomes by cells of one strain and the chemotactic response of those of the other.

The directional movement of plus cells towards minus cells in agar cultures of *Pleurotaenium* T_3 leaves little doubt that a chemotactic response is involved in this species and that the plus cells are moving along the diffusion gradient of an attractant secreted by the minus cells. Gradually, as this attractant permeates the whole agar medium the whole clone is stirred into increased activity.

Ling & Tyler (1974) observed that in *P. ehrenbergii* by *P. coronatum* crosses, *ehrenbergii* cells are relatively more active, several *ehrenbergii* cells congregating round a single *coronatum* cell. Further crosses between these two and especially in *P. coroniferum* have revealed that this "anisogamous" behaviour is regulated to some extent by the relative numbers of the two clones and that the previously passive clone can assume an active role when it is markedly superior in numbers. This would tend to suggest that the normally active clone also secretes an attractant.

Among algae chemotaxis has been demonstrated in *Sphaeroplea* (Pascher, 1931 - 1932), three species of *Fucus* (Cook et al, 1948; Cook & Elvidge, 1951), *Oedogonium* (Hoffman, 1960) and in the morphologically isogamous *Chlamydomonas moewusi* var. *rotunda* (Tsubo, 1961). Tsubo reported that plus gametes were attracted by minus gametes or their filtrate enclosed in a capillary tube. The reciprocal

test was negative.

It is probable that at least two hormones are secreted in *P. ehrenbergii*, one to initiate pairing, the other papillae formation. This would explain the behaviour of PE F_1 2, the minus clone that will pair but not conjugate with plus clones. Since it remains inactive in triple associations where the other two cells are actively conjugating, it probably cannot secrete nor respond to the second hormone.

The multiple cross results indicate without a doubt that selective mating occurs to a large extent in the *P. mamillatum* complex. In some cases the selective force is strong enough for cells of one clone to disrupt previously paired cells of other clones.

The selection appears to operate in two independent stages. The first is an active preferential selection of pairing partners, for example, a preference for partners nearest to the selector in size. Size is not the only factor. Often, one clone of a mixture of equal-sized minus clones will be the superior attractant and all plus clones will prefer it to all other minus clones.

The second stage is a passive, physiological kind of selection typified by cross 18. In this cross the results indicate a selection against the haploid *ehrenbergii* not for pairing, but for successful zygospore formation. There was no selective pairing (Fig. 46) but the diploid *ehrenbergii* produces more zygospores presumably because its time of gamete release synchronised with that of the diploid *P. coronatum* better than did the haploid *ehrenbergii*.

Crosses conducted suggest a fundamental difference between *P. ehrenbergii* and *P. coronatum* in that *ehrenbergii* prefers other

ehrenbergii to *coronatum* clones and vice versa. The extent of this difference is difficult to evaluate insofar as selective pairing occurs amongst *ehrenbergii* clones, that is, the *ehrenbergii* may be preferred to the *coronatum* simply because it is a very attractive clone! The situation is further complicated by the size factor and differences in activity of the various clones. However, a clear picture should emerge if a large number of both the haploid and diploid clones of both *ehrenbergii* and *coronatum* are crossed.

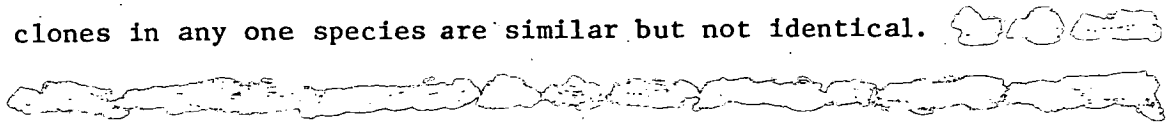
The acceptance of previously rejected cells when no preferred cells are available is truly remarkable. If anything, it indicates that differences in cell numbers between clones have to be extreme before established pairing preferences are overriden.

The author knows of no previous record of selective mating in the desmids, nor indeed the algae in general, but suspects that it may also occur in *C. botrytis*. Brandham (1967) studying an "anisogamous" mating behaviour in *C. botrytis* mentioned a plus clone (Group 2) that is passive with respect to one group (Group 1) of minus clones but active with respect to a second group (Group 3) of minus clones (Brandham, 1967; Fig. 2). It is probable that in a cross involving all three clones pairing between Group 1 x Group 2 will outnumber that between Group 2 x Group 3.

It seems plausible that the selection is mediated through either the quantity or quality of a hormone. The results point to minute but detectable differences in the attractant secreted.

The pairing observed between *Pleurotaenium* T_4, *P.* T_3 and *P.* WK 3 is difficult to explain. It may be that there is some similarity in

the attractants rather than the existence of a genetic relationship between the clones. The single clones involved in each instance further supports the view that the attractants secreted by different clones in any one species are similar but not identical.



The secretion of a mucilage envelope by conjugating cells appears to be common to many desmids. However, there is some variation in the actual conjugation process. In *Pleurotaenium*, the conjugation papillae originate from a cylinder of new wall material intercalated between semicells at the isthmus, and conjugation takes place in a preformed conjugation vesicle (Ling & Tyler, 1972a). Kies (1968) described for *M. papillifera* Breb. an identical type of conjugation and proposed the establishment of a *M. papillifera* type of zygospore formation in desmids. Whether the gametes are truly naked or are still bound by thin wall material remains to be seen, though the amoeboid gametes in *Pleurotaenium* (Ling & Tyler, 1972a) would suggest the former.

The process is essentially the same in *M. rotata* (Lenzenweger, 1968), *M. hardyi* and *M. laticeps*. In *M. hardyi* and *M laticeps* there appears to be a thin conjugation tube rather than a conjugation vesicle.

Another variation is seen in *C. botrytis* (Pickett-Heaps, 1975) and *St. orbiculare* var. *ralfsii* where the isthmus splits apart at one side and a papilla, apparently bounded by a thin wall, is produced. As the papillae touch the walls fuse to form a filmy conjugation tube.

The morphology of the *Pleurotaenium* zygospore is unusual in several ways. A significant feature is the operculum. Operculate zygospores were first reported for *P. ehrenbergii* and *P. trabecula* var. *mediolaevc*

by Ling & Tyler (1972a) who were optimistic that the operculum may prove a useful generic criterion of *Pleurotaenium*. Since then not only has it been found in all the other five *Pleurotaenium* species studied but also in *M. laticeps* and *M. hardyi*. The rim on the *Pleurotaenium* operculum indicates that it is an elaborate structure differentiated from exospore II. The *Micrasterias* operculum is relatively simple though in *M. laticeps* it appears to be a combined exospore-mesospore operculum. Though zygospores have been reported for several *Micrasterias* species and a detailed ultrastructural study made of the zygospore walls of *M. papillifera* (Kies, 1968), this is the first report of opercula in the genus. Unless the zygospores have undergone germination it would be quite difficult, especially where spines are present, to detect the presence of an operculum. Lenzenweger (1968) and Muller (1974), who observed germination in *M. rotata* and *M. papillifera* respectively, made no mention of it. It is likely that the opercula in *M. hardyi* and *M. laticeps* are structures differentiated at zygospore wall formation not merely dissolved out at the onset of germination. Unless specifically sought for, an operculum, especially of the *hardyi* and *laticeps* type, can easily be missed.

There is little doubt that the rimmed operculum is an important identifying character for *Pleurotaenium* and possibly so is the plain operculum for *Micrasterias*. If future research were to confirm the latter it would be invaluable in distinguishing *Micrasterias* from other genera, especially *Euastrum*, and settle whether plants like *Micrasterias moebii* (Borge) West & West are really *Micrasterias* or

Euastrum, that is, if the *Euastrum* zygospore is not operculate.

Zygospores of desmids are usually stated to have three walls (at the light microscope level) and *M. hardyi*, *M. laticeps* and *St. orbiculare* var. *ralfsii* are certainly no exceptions; but in *Pleurotaenium* five walls are normally discernible. Exospore I is perhaps equivalent or identical to the primary exospore on *M. papillifera* zygospores (Kies, 1968).

Another noticeable difference is the uniform absence of any external ornamentation on the *Pleurotaenium* zygospores in contrast to the spiny ones of other desmids, for example, *M. hardyi*, *M. laticeps* and *St. orbiculare* var. *ralfsii*.

The presence of mammillae on the mesospore is unique to *Pleurotaenium* zygospores. Previous descriptions of zygospores of *P. ehrenbergii* (Ralfs, 1848; West & West, 1904), *P. trabecula* (Ehrbg.) Nag., *P. tridentulum* (Wolle) W. West (West & West, 1904), *P. wallichianum* (Turn.) Krieger (Ramananthan, 1962) and *P. subcoronulatum* (Turn.) West & West (Ramanathan, 1963) made no mention of mammillations. Hardy (1906) and West (1909) described the mesospore of *P. ovatum* var. *tumidum* as papillate and from their figure (West, 1909) it is clear that it closely resembles that of the *Pleurotaenium* species presently studied. Bicudo (1969) and Ramanathan (1962), however described a smooth mesospore and spiny exospore for *P. ovatum* var. *tumidum* and Krieger (1937) described *P. minutum* (Ralfs) Delp. zygospores as globose, with rounded to conical processes.

It is doubtful whether West & West (1904) missed the mamillations on *P. ehrenbergii*, *P. trabecula* and *P. tridentulum*. Since all seven

Pleurotaenium species investigated here and also *P. ovatum* var. *tumidum* described by Hardy (1906) and West (1909) are of Australian origin one is led to the improbable conclusion that only Australian *Pleurotaenium* species have mammillate mesospores. Nevertheless, attempts were made to collect *Pleurotaenium* from overseas. An English sample contained *P. tridentulum* cells, but attempts at inducing conjugation in the raw sample and isolating clones were both unsuccessful. Other *Pleurotaenium* clones isolated from Macquarie Island, Munich, British Columbia and England have not shown any sexuality as yet.

Germination vesicles of desmids normally have a single wall, the endospore (Starr, 1959; Lippert, 1967). The same is true of *M. hardyi*, *M. laticeps* and *St. orbiculare* var. *ralfsii*. However, in *Pleurotaenium* the germination vesicle always has two walls. Furthermore, the germination vesicle in *Pleurotaenium* is more conspicuous because of its yellow colour and abundance of haematochrome, indicating, perhaps, a preponderance of certain pigments occurring in lesser quantities or being deficient in other desmids.

The first detailed account of meiosis in the desmids was made by Brandham & Godward (1965a) working with *C. botrytis*. In this species, the zygospore nuclei did not fuse until immediately before germination. The observation on non-fusion meiosis supports the view that this too

is the case in *Pleurotaenium* where zygospores germinate at a stage of meiosis (diplotene or diakinesis) roughly between that of *C. botrytis* var. *botrytis* (fusion nucleus) and *C. botrytis* var. *tumidum* (metaphase I). A significant difference between meisois in *Pleurotaenium* and that reported for *Cosmarium* is the interchromosomal stickiness of the former. Strongly adherent chromosomes have also been reported in desmid mitoses (King, 1960; Brandham & Godward, 1965a), where chromosomes are invested with a stainable matrix, possibly of nucleolar origin (Fowke & Pickett-Heaps, 1969; King, 1959; Ueda, 1972).

An asynchronous bivalent dissociation and anaphase is a feature of *C. botrytis* (Brandham & Godward, 1965a). Both synchronous and asynchronous anaphases were observed in *Pleurotaenium*.

King (1960), Brandham & Godward (1965a) and Ueda (1972) have shown that some desmid chromosomes are polycentric. King, however, reported a normal disjunction of mitotic chromatids in *Mesotaenium caldariorum*, presumably implying that the chromosomes had normal centromeres. Polycentric chromosomes have been well demonstrated in *Zygnema* (Bech-Hansen & Fowke, 1972), but *Spirogyra* has a well-defined, single localized centromere (Mughal & Godward, 1973). The median to sub-median constrictions of metaphase II chromosomes of *Pleurotaenium* are indicative of localized centromeres, as is the flexure of the arms at the centromere in anaphase I (Fig. 9) and the reductional separation of the centromeres at anaphase I.

The chromosome numbers of various desmids are known and except for one, are based on mitosis (King, 1960; Brandham, 1965b; Godward, 1966; Kasprik, 1973). King found considerable variation in

chromosome number within the same species, in *C. botrytis* and *Netrium digitus*, and within the same clone, in *C. cucumis*. This coupled with the existence of a polycentric chromosome organization led King to suggest chromosome fragmentation or fusion as a cause of the variation. Fragments of polycentric chromosomes would not be acentric and consequently lost but would segregate normally in mitosis.

Brandham (1965b) finding similar inter- and intraspecific variability in chromosome number concluded that a combination of polyploidy, aneuploidy and fragmentation of chromosomes together with other mitotic irregularities could give almost any chromosome number in a desmid species, and might make it unlikely that any two clones obtained from different localities would have the same chromosome number.

In *Pleurotaenium* the opposite is true. Not only do clones (e.g. *P. mamillatum*) from widely separated localities have the same chromosome number, but morphologically different clones (e.g. *P. coroniferum*) were found to have the same chromosome number also. Perhaps this is a direct result of *Pleurotaenium* having localized centromeres in contrast to the polycentric chromosomes of most other desmids.

Previous records of chromosome numbers in the genus *Pleurotaenium* are n = 104 for *P. trabecula* (King, 1960) and n = 24 for *P. minutum* (Nizam, 1960). The counts of n = 53 for *P. mamillatum*, n = 52 for *P. trabecula* var. *mediolaeve*, n $\doteq$ 51 for *P. coroniferum* and n = 49 $\pm$ 1 for *Pleurotaenium* T_4 probably represent true haploid complements. The small difference in chromosome number suggests perhaps a close relationship between the four species but results from crosses have so far not borne this out. Though morphologically distinct bivalents

were consistently observed in *P. mamillatum* and *P. trabecula* var. *mediolaeve* further research is required to establish their significance or otherwise.

Another interesting, and inexplicable, aspect of the chromosome numbers of the *Pleurotaenium* species is that the observed numbers are all very near or are simple multiples of 50; or 25, if the n = 24 for *P. minutum* (Nizam, 1960) is included.

Pleurotaenium T_3 is problematical. Its morphological resemblance to *P. trabecula* var. *mediolaeve* and its chromosome number of n = 100 suggests that it could be a diploid of var. *mediolaeve* (n = 52). However, experience with other *Pleurotaenium* species indicates that if this were the case, it should be about twice the size of var. *mediolaeve* and interfertile with it. On both these criteria *Pleurotaenium* T_3 is set apart from *P. trabecula* var. *mediolaeve*.

In *M. laticeps* the small size and large number of chromosomes present a major barrier towards a detailed study of the meiotic process. Kasprik (1973) obtained a mitotic count of 114/119 for *M. laticeps*. No accurate counts were made of Australian strains but results indicate that the chromosome number is of the same order of magnitude.

Getting *M. hardyi* zygospores to germinate regularly may be a problem. However, the chromosome number is small and should not pose any forseeable difficulty.

Ueda (1972) has shown that the chromosomes in *M. americana* are polycentric. There is a suspicion that the chromosomes in *M. hardyi* and especially *M. laticeps* are similarly organised.

In the placoderm desmids two gones usually result from meiosis of the zygospore (Fritsch, 1948; Starr, 1959; Lippert, 1967; Lenzenweger, 1968). The formation of two gones in *M. laticeps*, *M. hardyi* and *St. orbiculare* var. *ralfsii* further confirm this generalisation. However, there are exceptions. *Hyalotheca dissiliens* (Pothoff, 1927), *C. biretum* (Starr, 1959), and *M. papillifera* (Muller, 1974) have single survivors of meiosis, and it seems the single gone is a generic character of *Pleurotaenium*.

Apparently, in cases where both the number of chromatophores in the germination vesicle and the number of products are known, there is a correlation between the chromatophore-product numbers. For example, in *C. biretum* (Starr, 1959) and the various *Pleurotaenium* species where there is normally one chromatophore in the germination vesicle, a single product results; in *C. turpinii* (Starr, 1955a), *Cl. moniliferum* and *Cl. ehrenbergii* (Lippert, 1967), *M. laticeps* and *M. hardyi* where there are two chromatophores, two gones are produced. Biebel (1964) observed 2, 3 or 4 chromatophores per spore in *Netrium digitus* var, *digitus* and reported that 2 products are most common, though 1, 3, 4, and 5 products are not uncommon.

St. orbiculare var. *ralfsii* normally has two chromatophores with the resultant two gones. However, in this species occasional vesicles with 3 and 4 chromatophores have produced 3 and 4 gones respectively. Thus there is good evidence for a simple linear relationship between the number of chromatophores in the germination vesicle and the number of gonal products.

Production of a single large gone in *M. laticeps* is unusual.

Brandham (1965a) observed similar products in *St. denticulatum* and presumed they gave rise to diploids which he subsequently isolated from the same culture. The surprising mode of division of the *laticeps* gone leaves little doubt as to its binucleate nature.

There is no doubt that the large-celled clones arising in the various *Pleurotaenium* cultures are true diploids. Brandham (1965b) has pointed out that chromosome numbers of desmids with polycentric chromosomes can vary considerably as a result of fragmentation, but in *Pleurotaenium* the chromosome counts indicate a straight doubling.

Brandham (1965a) suggested that diploids, in the desmids, are formed by either a non-disjunction of chromatids or the displacement of the dividing nucleus followed by fusion of the daughter nuclei. It is very likely that diploids in the various *Pleurotaenium* and *Micrasterias* species studied are similarly formed. In *Pleurotaenium* T_4 one of the diploid clones probably results from the survival and subsequent fusion of two sister nuclei from meiosis.

Diploids or presumed diploids have previously been reported in *Micrasterias* (Kallio, 1951), *Cl. siliqua, C. botrytis, St. denticulatum, St. dilatatum* (Brandham, 1965a) and *C. turpinii* (Starr, 1958). All these diploids were either artificially induced or else appeared spontaneously under culture conditions. As yet there is no authenticated case of naturally-occurring true diploids though Brandham (1965a) suspects that such may exist.

The frequent occurrence of diploids in culture may be attributed to the comparatively immense number of cells that are produced in culture and or culture conditions favourable to the production and

survival of diploids, though there is no supporting evidence. The coddling required by the diploids, especially in the initial stages, is indicative of their vulnerability. In the wild they probably succumb before they have a chance to stabilize and multiply.

It has been shown that concomitant with diploidy the volume of the desmid cell doubles and the shape of the cell increases in complexity; a biradiate changes to a 3-radiate, a 3-radiate to a 4-radiate (Waris & Kallio, 1964; Brandham, 1965a). As yet the effects of the sexual cycle on this increase in radiation is not known. Even though triploid and tetraploid zygospores formed by these diploid strains with other compatible haploid and diploid strains have been induced to germinate no viable offsprings were recovered (Starr, 1958; Brandham, 1965a).

In *Pleurotaenium* the effects of diploidization are evident in an increase in cell size and in the number of apical tubercles. The cell volume increases are passed on to the offspring, the 3n and 4n zygospores producing F_1s that are intermediate and large in size respectively. Unfortunately it was not possible to study the effects of the sexual cycle on radiation because the *Pleurotaenium* are omniradiate, an increase in ploidy having no effect on radiation.

The large cells of *M. laticeps* and *M. hardyi* are about twice the size of the normal cells and have increased in radiation. They retain their large size. Thus there is sufficient evidence to regard them as diploids.

Compared to the *Pleurotaenium* species the much greater variation in the F_1s of diploid x haploid *M. laticeps* crosses is expected because

of the greater morphological complexity of *laticeps*. Another reason may be that the combined chromosomes in *laticeps* may not be as evenly distributed to the daughter nuclei as in the *Pleurotaenium* species.

It has not been possible to observe the shape assumed by the new semicells at the first division of gones from a 4n zygospore in *M. laticeps* because only plus mating diploid clones have been isolated. Of the many possibilities such as biradiate, 3-radiate, 4-radiate and combinations of these, the results from the initial isolation of the diploid clones suggest that the biradiate with an odd 3-radiate would be the most likely but that eventually the dominant shape in the clones would be 3-radiate. If future research were to confirm this it could be a significant factor in indicating natural diploidy, especially in the multi-radiate genus *Staurastrum*; products from the zygospores initially producing new semicells that are one radiation lower than that of the parents.

Like many other aspects of *Pleurotaenium* in general, the tapered character in *P. ehrenbergii* is also difficult to resolve. There is no doubt that the trait is passed on to the offspring. The puzzling part is that this inheritable trait should show a tendency, in both the haploid and derived diploid, to revert to normal after an extended period of apparent stability.

In *P. coroniferum* the cause of the morphological differences between W 1 and T 1 is likewise difficult to gauge. Their stability under culture conditions rule out environmental factors. The

chromosome numbers of the two clones are apparently the same. Where there is a large complement of small chromosomes exact counts are difficult. The cause could be a very small disparity in chromosome numbers between W 1 and T 1 with the F_1 having the average of the two chromosome numbers and an intermediate shape. Clearly, further research in the form of backcrosses, mitotic counts, etc. are required.

Lippert (1967) observed a similar situation in *Cl. moniliferum* where clones of different sizes produce intermediate F_1s. Rough chromosome counts suggest both parents and F_1s have the same number of chromosomes.

Observations on the 3-radiate clones in *M. laticeps* agree, more or less, with the findings of Brandham & Godward (1964) that the small 3-radiate form in *C. botrytis* arises from gones and that the 3-radiate character is not under any kind of genic control.

Starr (1958) observed that the small 3-radiate form of *C. turpinii* arose from a single gone produced by a germinating zygospore, the normal number of gones being two. The small 3-radiate clone in *M. hardyi* probably arose likewise.

Waris (1950) and Kallio (1959) have shown that the old semicell exerts an influence on the young developing semicell in *Micrasterias*, even in enucleate cells. They attribute this influence to the "cytoplasmic framework" of the old semicell. Brandham & Godward (1964) have used this concept as the basis of an explanation of the 3-radiate form in *C. botrytis*. They suggested that the 3-radiate form is caused probably by the formation of an atypical "cytoplasmic

framework" either just before or concurrently with the first vegetative division of a gonal cell. It is maintained by the influence of the "cytoplasmic framework" of the old semicell upon the young semicell. Reversion to normal could be explained by a gradual change in the framework under the influence of the nucleus.

The uni-radiate and *berganii* shape in *M. hardyi* is probably controlled by culture conditions.

The *berganii* shape in *M. hardyi* has not been reported from the wild. Tyler (1970) first observed it in culture but made no mention of its stability. Single isolations have shown it to be unstable, nevertheless the short-lived, single incidence of the exclusive production of *berganii* semicells in a new media indicate its apparent stability under a particular set of media and perhaps culture conditions.

Here, as well as in the *alata* semicell situation, the specific names for the semicell shapes are merely descriptive, there is no implication of a *M. berganii - M. hardyi - M. alata* relationship.

Of all the morphological variants in *M. hardyi*, clone WL 1 m most approaches a mutant, rather than a polymorphic or ephemeral variation. If F_1s can be successfully produced this clone should offer a potential for genetic research.

It is difficult to arrive at definite conclusions about these morphological changes in culture. Their usually transient nature renders experimentation difficult. The problem probably has its origins in the culture media of desmid agar with soil extract and soil-water where fluctuations in quality are imposed by the soil used,

temperature, and bacterial or fungal microflora.

Classification based on morphology under controlled conditions have become quite commonplace in other groups of algae. Goldstein (1964), Coleman (1959) and Stein (1966) have compared growth and morphology of various strains of *Volvocales* under culture conditions and suggested a form of classification based on culture morphology.

Attempts were made to culture pure clonal strains of desmids in a chemically defined inorganic medium, but growth was poor. Further research is required.

The results from the *P. mamillatum* complex form the most significant part of this investigation primarily because of their important taxonomic implications. Foremost is the question of the relationship of the Goulburn Weir *P. coronatum* to *P. ehrenbergii*. The integrity of the karyotype is demonstrated by its occurrence in opposite mating type at Glenmaggie, 200 km. away in a different catchment. (It must be borne in mind though, that the nature and origins of these complex genetic factors which we call mating types is not well understood.)

The question of the ploidy level of *P. coronatum* is also open. The two instances of non-fusion meiosis in PCGW 1 x PCGM 1, with chromosomes of a single nucleus associated mostly as bivalents, suggest a basic polyploidy. However, the morphological findings argue against classifying it as a polyploid *P. ehrenbergii*.

In each of the *Pleurotaenium* species studied the number of apical tubercles lies in a narrow and fixed range and an increase in ploidy

is always accompanied by an increase in cell size and in the number of tubercles. The haploid *P. ehrenbergii* (n = 53) has 7 - 10 tubercles, the diploid 11 - 15 tubercles. If *P. coronatum* (n = 146) is a true polyploid of *P. ehrenbergii* it would be expected to be larger and have more tubercles than the diploid *P. ehrenbergii*, but not only is it slightly smaller it also has only 8 - 11 tubercles.

Assuming that *P. coronatum* is a species distinct from *P. ehrenbergii* the remarkable success at hybridization poses a major problem. But if *P. coronatum* were a naturally occurring diploid of some *Pleurotaenium* then hybridization would be enhanced; polyploidy buffering genotypes against the shock of absorbing foreign genomes (de Wet, 1971).

Without exception the triploid zygospores of all the *Pleurotaenium* species studied are remarkably viable, there is thus good reason to expect comparable viability in hybrid zygospores. Perhaps in these *Pleurotaenium* species the actual chromosome numbers are of little consequence, what matters is the possession of a basic set, an intact haploid complement of chromosomes. Again assuming that *P. coronatum* is diploid this explanation holds for all the crosses and in the cross haploid *P. ehrenbergii* x *P. coronatum* the F_1 have a basic *P. coronatum* set. However, one still wonders at the success of the cross *P. ehrenbergii* (n = 53) x PCGW 1 2n where n probably = 292!

There is little doubt that all strains in the *P. mamillatum* complex are closely related genetically. The problem lies in the taxonomy. In previous papers Ling & Tyler (1972a, 1974) identified strains of *Pleurotaenium* from Tasmanian lakes, and from Yan Yean

Reservoir and Goulburn Weir, both in Victoria, as *P. ehrenbergii*, *P. mamillatum* and *P. coronatum* respectively. No doubt surrounds the identification of *P. mamillatum* - the material, from the type locality, agrees in every way with the original description (West, 1909). The position with the two other taxa is dubious.

Bourrelly (personal communication) points out that the *P. ehrenbergii* and *P. coronatum* strains differ from the European ones of his experience. There, they always have rounded tubercles whereas in Australian forms they are conical and pointed. Bourrelly based his opinion also upon de Brebisson's original figures and exsiccate material of *P. coronatum*. Through the courtesy of Professor Bourrelly the author has examined this material and fully agrees with him. Also a strain of *P. coronatum* was isolated from a sample kindly sent by Dr. K. Handke from Munich. Bourrelly (personal communication) accepts this strain as *P. coronatum* (Breb.) Rabenh. It will not cross with any of the putative *P. coronatum* strains from Australia. Bourrelly's contention is that all three taxa considered by Ling & Tyler (1974) belong to the species *P. mamillatum*. The results show that two of the taxa certainly do so; all three are closely related genetically, but in a complicated way, and not for the reasons proposed by Bourrelly.

The disturbing fact is that relying entirely on comparative iconography with published works (as one often must in Tasmania) then the strains could quite legitimately be identified as separate species, indeed, except for the intercrossing results, there is every justification for identifying the strains as separate species. Even using only the figures of well-respected doyens of desmidiology, there is

no difficulty in finding iconographic matches. The *ehrenbergii* strain closely resembles the figure by Irenee Marie (1939, Pl. 11, Figs. 5, 6 which shows conical tubercles), and also agrees with West & West (1904, Pl. 29, Figs. 9 - 11) except for the rounded tubercles in their figure. However, in the text (p. 206) the Wests describe *P. ehrenbergii* as ".... apices truncate bordered by a ring of conical or rounded tubercles ....". Scott & Prescott (1958, Fig. 2(2)) show *P. ehrenbergii* from Arnhem Land with conical tubercles, as do Prescott & Scott (1952, Fig. 2(3)) for South Australian material. The confusion in *P. coronatum* has already been pointed out (Ling & Tyler, 1974). Furthermore, West (1909) reported *P. coronatum* (Breb.) Rabenh. from Yan Yean Reservoir, Victoria. A careful search of several Yan Yean and other Victorian samples has not revealed a single *P. coronatum* (Breb.) Rabenh., but the Goulburn Weir strain of *P. coronatum* has been found in four different Victorian localities over three separate catchment areas.

Other workers, in other parts of the world, are likely to be faced with the same problem of the iconotype, and the bewildering array of described taxa in the genus *Pleurotaenium*. There is no doubt the strains in the *mamillatum* complex are closely related. For convenience alone the specific names for the various strains have been retained.

From a taxonomic point of view the *P. mamillatum* complex neatly fits Mayr's (1963) biological species concept of groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups. The other *Pleurotaenium* and

Micrasterias species similarly qualify as biological species, the members in each group (both haploids and diploids) interbreeding remarkably well with each other and clearly reproductively isolated from other groups.

The ease with which strains of widely different chromosome number and probable ploidy level can be crossed (e.g. *P. ehrenbergii*, n = 53 x PCGW 1 2n where n probably = 292), and the considerable viability and fertility of resulting offspring is in marked contrast to that reported for some other desmids (Brandham, 1965a; Starr. 1958), Volvocales (Coleman) and Characeae (Proctor, 1975). In the strains studied, cells put up with any chromosome number above the haploid with apparent impunity. The results of crosses between clones from widely separated areas indicate that at least sexual, if not genetic, compatibility, in the *P. mamillatum* complex, *P.* T_3, *M. hardyi* and *M. laticeps* extends over much wider geographical areas than reported for other algal groups (Coleman; Proctor, 1975). This free fertility between local and increasingly disjunct populations support the current view that long-distance dispersal is generally effective in maintaining gene pool cohesiveness, and that many species are essentially cosmopolitan in their distribution (Proctor, 1975). Here Proctor's (1959, 1966) findings of the successful dispersal of desmids by waterbirds has relevance.

However, sexual compatibility should be interpreted with care. Proctor (1975) found that *Chara zeylanica* populations from various parts of the world are able to cross to the extent of oospore formation, but these oospores are non-viable. In other words, mating

compatibility is not matched by equivalent genetic compatibility. Proctor (1970, 1975) in his work on Charophytes stressed that true genetic compatibility associated with potential gene flow obviously can only be gauged by studying the progeny of backcrosses of F_1 generations to both parental types.

Few backcross results are available for the species studied, but the large number of zygospores and successful germination of zygotes producing fertile F_1 progeny are proof of successful genetic exchange.

A major hurdle is the difficulty of performing all the necessary crosses and testing zygospore viability. In the *P. mamillatum* complex alone the number of crosses that can be done rises in geometrical progression. As a corollary, the potential for genetical research is also immense.

It can also be argued that the small number of clones from each locality may not be truly representative of the population. In several cases the small number of clones used was unavoidable because of the scarcity of plants of the particular species from any one sample. Secondly, the isolation, culture, maintenance, crossing and eventual characterisation of clones is a time consuming process. Hence, potential sexuality by inference is, to a certain extent, justified.

With the characterisation of species biologically we have, at last, a point of reference from which to proceed. New strains from nature can be tested for sexual compatibility with ones maintained in culture and their taxonomic content determined objectively.

The results also have strong taxonomic connotations at the generic level. Although only a few species of *Pleurotaenium*, and from a narrow geographical range, were studied experimentally, the results leave little doubt as to the homogeneity and reality of the genus *Pleurotaenium*. The results also indicate that other desmid genera, such as *Micrasterias*, are also of taxonomic reality, though their precise limitations are as yet uncertain.

The genus *Pleurotaenium* merges with *Penium*, *Cosmarium* and *Actinotaenium*. It differs from *Docidium* in that cells in the latter have axile or central chloroplasts and are plicate at the base of the semicells. *Micrasterias* merges imperceptibly with *Euastrum*. The sexual features of some species of *Penium*, *Cosmarium* and *Euastrum* are known (West & West, 1904, 1905, 1908; Krieger, 1937) but apparently there is no feature that is characteristic of any of the genera. To date no sexual features of *Docidium* are known.

The *Pleurotaenium* strains used in this investigation are straightout *Pleurotaenium*. There is no uncertainty about their being classified as other desmid genera. Similarly, *M. hardyi* and *M. laticeps* are easily recognisable as *Micrasterias*. In short, the crucial test has yet to be made, that is whether sexual characteristics can effectively deal with borderline cases, the central problem in desmid taxonomy. The author believes it can. For example, both Krieger (1937, Taf. 39, Fig. 3) and Prescott et al (1975, Pl. XXXVIII, Fig. 12) figure *P. minutum* (Ralfs) Delp. zygospores as spherical with conical protuberances, quite unlike the zygospores of the

Pleurotaenium species investigated. The morphology of the semicells of *P. minutum* are also atypical of *Pleurotaenium* and borders on that of *Penium*. On these grounds it is suggested that *P. minutum* is erroneously classified as a *Pleurotaenium* species.

Another case in point is *St. orbiculare* var. *ralfsii* which has spiny, cratered zygospores. Spiny, cratered zygospores identical to those of *St. orbiculare* var. *ralfsii* were observed in a desmid (currently under culture) which has the morphological features characteristic of the genus *Staurodesmus*. Teiling (1967) created the genus *Staurodesmus* to include the smooth-walled, monospinous species previously under *Staurastrum* and *Arthrodesmus*. *St. orbiculare* var. *ralfsii* resembles a *Staurodesmus* except that it lacks spines.

The author joins Cook (1963) in proposing that sexual characteristics are essential features in the identification of desmid taxa.

A tentative plan, incorporating only the sexual features, for *Pleurotaenium, Micrasterias* and *Staurodesmus* is set out below.

PLEUROTAENIUM

Conjugation takes place in a preformed conjugation vesicle. Gamete escape pore in wall of lengthened isthmus. Smooth zygospores with 5 walls consisting of 2 exospores, 1 mesospore and 2 endospores; rimmed operculum, mammillate mesospore. Germination via operculum, released protoplast enclosed by 2 endospores. Conventional chromosomes. Single germination product.

MICRASTERIAS

Conjugation takes place in a conjugation vesicle or tube. Gamete escape pore in wall of lengthened isthmus. Spiny zygospores with 3 walls, exospore, plain mesospore and endospore. Simple operculum. 2 germination products, in some species one only.

STAURODESMUS

Spiny, cratered zygospores, 3 walls. No operculum. 2 germination products.

There is insufficient information from other genera. Cook (1963) made a detailed study of the sexual features of several *Closterium* species but no unifying characteristic at the generic level was evident. Though zygospores of several desmids are known, the results from this investigation suggest that the descriptions may not be totally reliable.

There is no suggestion of abandoning the morphological characteristics of these genera. The results presented here do not invalidate our understanding of the desmid taxa based on morphological criteria, they actually consolidate it. Initially our taxonomic determinations must, of necessity, be morphological, but it is believed that sexual criteria will be crucial in sorting out borderline cases.

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