

SEXUAL PROCESSES IN THE LIFE CYCLE OF *GYRODINIUM UNCATENUM*  
(DINOPHYCEAE): A MORPHOGENETIC OVERVIEW<sup>1</sup>

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ABSTRACT

*Sexual processes in the life cycle of the dinoflagellate Gyrodinium uncatenum Hulburt were investigated in isolated field populations. Morphological and morphogenetic aspects of gamete production, planozygote formation, encystment, excystment, and planomeiocyte division are described from observations of living specimens, Protargol silver impregnated material and scanning electron microscope preparations. The sexual cycle was initiated by gamete formation which involved two asexual divisions of the vegetative organism. Gametes were fully differentiated following the second division and immediately capable of forming pairs. Either isogamous or anisogamous pairs were formed by the mid-ventral union of gametes. Gametes invariably joined with flagellar bases in close juxtaposition. Complete fusion of gametes required ca. 1 h, involved plasmogamy followed by karyogamy and resulted in a quadriflagellated planozygote. Planozygotes encysted in 24–48 h to yield a hypnozygote capable of overwintering in estuarine sediments. Hypnozygotes collected from sediment in late winter readily excysted upon exposure to temperatures above 15° C. A single quadriflagellated planomeiocyte emerged from the cyst and under culture conditions divided one to two days later. The four flagella were not evenly distributed at the first division and both bi- and tri-flagellated daughter cells were formed.*

*Key index words:* encystment; excystment; gamete; Gyrodinium uncatenum; hypnozygote; planomeiocyte; planozygote

The existence of a sexual phase in the life cycle of dinoflagellates, while a source of controversy for some time, has in the past two decades been well documented in numerous species. Much of this effort has focused on identifying various developmental stages and characterizing the sexual cycle (von Stosch 1965, 1973, Cao Vien 1967, 1968, Zingmark 1970, Pfister 1975, 1976, 1977, Turpin et al. 1978, Walker and Steidinger 1979). However, several investigations have employed cytological and ultrastructural techniques to examine specific aspects of the sexual process in greater detail (Skoczylas 1958, von Stosch 1964, 1972, Spector et al. 1981, Chapman et al. 1981, 1982). With rare exception (Bibby and Dodge 1972, Chapman et al. 1982), knowledge of dinoflagellate sexuality has been derived from observations of cultured organisms. The lack of comparable observations for field populations may, as suggested by Chapman et al. (1982), result from the sexual process being a short lived phenomenon or occurring in only a few cells at any given time.

The recognition of a resting stage (hypnozygote)

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as part of the sexual cycle stimulated additional interest in dinoflagellate sexuality. The significance of hypnozygotes as seed populations for establishing dinoflagellate red tide blooms is recognized (Anderson and Wall 1978, Anderson and Morel 1979), and attempts are being made to identify environmental factors which stimulate development of various sexual stages (Anderson 1980, Watanabe et al. 1982, Pfister and Anderson 1984).

Recently, we reported the effect of hydrography upon the deposition of *Gyrodinium uncatenum* cysts in estuarine sediments (Tyler et al. 1982) and have examined factors effecting encystment in culture (Anderson, unpublished observations) and germination of seed bed populations (Tyler, unpublished observations). Here we report on morphological and morphogenetic aspects of sexuality in isolated field populations of *G. uncatenum*.

#### MATERIALS AND METHODS

To examine events leading to encystment, a 57 L Nalgene cylindrical tank was filled with water taken by bucket from a surface *Gyrodinium uncatenum* bloom located in Baltimore Harbor, Maryland (39°15' N; 76°34' W). The population was isolated at 09:30 h August 18, 1980 and held on deck where it was exposed to the natural light regime and maintained at ambient temperature (ca. 26° C) by continuously bathing the outside of the tank with baywater. The isolated population was thoroughly mixed immediately prior to sampling by repeatedly thrusting the broad end of a 2 L graduated cylinder toward the bottom of the tank. This method of agitation simulated shaking, avoided the production of a large vortex as in stirring and resulted in an even dispersal of organisms. Samples were arbitrarily drawn from the upper half of the tank at 30–60 min intervals for 48 h and then less regularly for an additional 52 h. At each sampling interval, 20 mL aliquots were preserved in a modified Bouin's fixative (Coats and Heinbokel 1982) and returned to the laboratory for subsequent staining by either the Bodian protargol silver technique (Tuffrau 1967) or a regressive alum hematoxylin procedure (Galigher and Kozloff 1971). Estimates of cell concentrations were obtained by counting the individuals present in single 1-mL aliquots of preserved samples. Percentages of dividing cells and fusing gametes were determined from hematoxylin stained material. The first 500 individuals encountered in each sample were tallied as nondividing cells, dividing cells or fusing gametes. Early and late developmental stages were given equal weight and thus even "figure-8" stages, whether dividing cells or fusing gametes, were counted as single individuals. The percentage of cells as planozygotes was similarly derived from Protargol preparations with 100 individuals being enumerated for each sample. Stained specimens were examined and photographed on a Zeiss microscope equipped with phase contrast optics and an Olympus OM-2N camera. Cell measurements were made of Protargol stained specimens using a calibrated ocular micrometer.

Excystment in *Gyrodinium uncatenum* was investigated using hypnozygotes isolated from sediment at ca. 4° C during February 1982. Sediment from Parish Creek (38°50'44" N; 76°30'31" W) was collected using a Petersen dredge and cleaned by the method of Wall and Dale (1968). Sediment was disaggregated by pulse sonication for 1 min using a Branson Sonifier Cell Disrupter 200 that delivered 30 W at 0.5-s intervals. Hypnozygotes were collected by sieving the resulting slurry through 64–20 µm Nitex nylon screens. Cleaned hypnozygotes were transferred to 15‰ "f/2" phytoplankton growth medium (Guillard and Ryther 1962) producing a 2 L "culture" of ca. 100 *G. uncatenum* cysts mL<sup>-1</sup>.

The "culture" was incubated at 20° C on a 12:12 h light-dark

cycle using General Electric Cool-White fluorescent bulbs with light intensity of 100 µE · m<sup>-2</sup> · s<sup>-1</sup>. Twenty-milliliter aliquots were taken at varying intervals and processed as described above.

Observations of cultured *Gyrodinium uncatenum* were of a strain isolated from the Potomac River in the summer of 1979 and maintained at room temperature (20–23° C) on 15 and 30‰ "f/2" phytoplankton growth media. The isolate was unialgal but not axenic or clonal.

For scanning electron microscopy, organisms were preserved in a variety of solutions with osmium tetroxide-aqueous mercuric chloride fixative (Parducz 1967) producing the best results. Preserved specimens were transferred through multiple distilled water washes, freeze dried using a Pearse-Edwards tissue dryer, coated with a gold-palladium alloy and examined on an AMR 1000A scanning electron microscope.

#### RESULTS AND DISCUSSION

*Sexuality and encystment.* To determine the developmental events associated with encystment, *Gyrodinium uncatenum* from a bloom was transferred to a large container and routinely monitored for several days. Subsequent to capture, a large proportion of the *G. uncatenum* cells began the sexual life cycle phase, thus permitting close examination of morphological and morphogenetic aspects of sexuality. At the time of isolation (T<sub>0</sub>), *G. uncatenum* numbered ca. 750 · mL<sup>-1</sup> and resembled the species description of Hulbert (1957). Protargol stained specimens averaged 33 × 29 µm with individuals ranging through a two-fold difference in size (see Table 1). Trophic organisms possessed a spherical to slightly ovoid nucleus with condensed chromosomes and generally two (range 1–5) nucleoli (Fig. 1). Cells had the typical dinoflagellate complement of two flagella with the striated strand and flagellar bases (= basal body) also staining deeply (Figs. 1, 2, 8, 9).

Early in the experiment, *G. uncatenum* increased significantly in size and had more than doubled in volume by T<sub>15</sub> (see Table 1). Other than their larger size, T<sub>15</sub> cells were indistinguishable from those of T<sub>0</sub>. During this period, *G. uncatenum* reproduced asexually and numbered ca. 930 · mL<sup>-1</sup> at T<sub>15</sub>. Dividing cells were infrequently encountered between T<sub>0</sub> and T<sub>15</sub> and never comprised more than 3% of any sample. In the following ten hours (T<sub>15</sub>–T<sub>25</sub>), the abundance of dividing cells was considerably higher and Protargol staining revealed the division sequence summarized in Figure 12. The first indication of division was the replication of flagellar bases resulting in four basal bodies arranged in two pairs (Fig. 12b). Elaboration of new flagella ensued immediately after basal body replication and was completed by mid-division. As cytokinesis proceeded, flagellar bases moved apart and each daughter cell received one old and one new basal body-flagellum complex (Figs. 3, 4, 12c–e). Nuclear and nucleolar elongation accompanied flagellar separation, and mitosis occurred as described in other free-living dinoflagellates (Dodge 1963). By late division, cells were loosely attached at the epicone and flagellar bases of the two daughter cells were widely spaced (Figs. 5, 12f).

TABLE 1. Cell size and nuclear characteristics of developmental stages associated with encystment in the isolated *Gyrodinium uncatenum* bloom population.<sup>a</sup>

		Somatic			Nuclear			Nucleoli		Cell volume <sup>b</sup> ( $\times 10^4 \mu\text{m}^3$ )
		L	W	n	L	W	n	No.	n	
Trophic cells ( $T_0$ )	$\bar{X}$	33.3	28.6	30	13.1	10.5	30	2.1	30	1.4
	Range	24.7–40.2	21.6–36.1		9.3–15.4	7.2–15.4		1–3		
	S	3.67	3.68		1.56	2.08		0.55		
	SE	0.67	0.67		0.28	0.38		0.10		
Pre-division trophic cells ( $T_{15}$ )	$\bar{X}$	49.2	42.0	30	14.2	15.3	30	2.2	30	4.5
	Range	36.0–64.9	31.9–56.6		11.3–18.5	11.3–18.5		1–3		
	S	7.01	6.02		1.48	1.81		0.55		
	SE	1.28	1.10		0.27	0.33		0.10		
Gametes ( $T_{27}$ )	$\bar{X}$	31.2	21.4	60	12.4	9.8	60	2.1	35	1.0
	Range	21.6–41.2	13.4–28.8		8.2–20.6	7.2–11.3		1–4		
	S	4.34	3.29		1.78	0.93		0.47		
	SE	0.56	0.43		0.23	0.12		0.08		
Planozygotes ( $T_{35}$ )	$\bar{X}$	43.4	38.7	30	15.1	13.9	30	4.2	24	3.4
	Range	33.0–50.5	27.8–45.3		13.4–17.5	11.3–17.5		3–6		
	S	4.71	4.49		1.10	1.75		0.83		
	SE	0.86	0.82		0.20	0.32		0.17		
Planozygotes ( $T_{56}$ )	$\bar{X}$	44.6	37.0	30	14.7	13.3	30	4.8	30	3.2
	Range	38.1–50.5	28.8–46.4		12.4–16.5	11.3–15.4		3–7		
	S	3.67	4.33		0.93	0.93		0.99		
	SE	0.67	0.79		0.17	0.17		0.18		
Newly formed cysts ( $T_{100}$ )	$\bar{X}$	48.2	38.8	30		9.5 <sup>c</sup>	30	4.1	30	
	Range	41.2–54.6	33.0–44.3			8.2–11.3		3–6		
	S	3.94	3.07			0.80		0.71		
	SE	0.72	0.56			0.15		0.13		

<sup>a</sup> All observations are of Protargol silver stained specimens.

<sup>b</sup> Cell volumes were approximated using the formula for a prolate spheroid and mean length-width measurements.

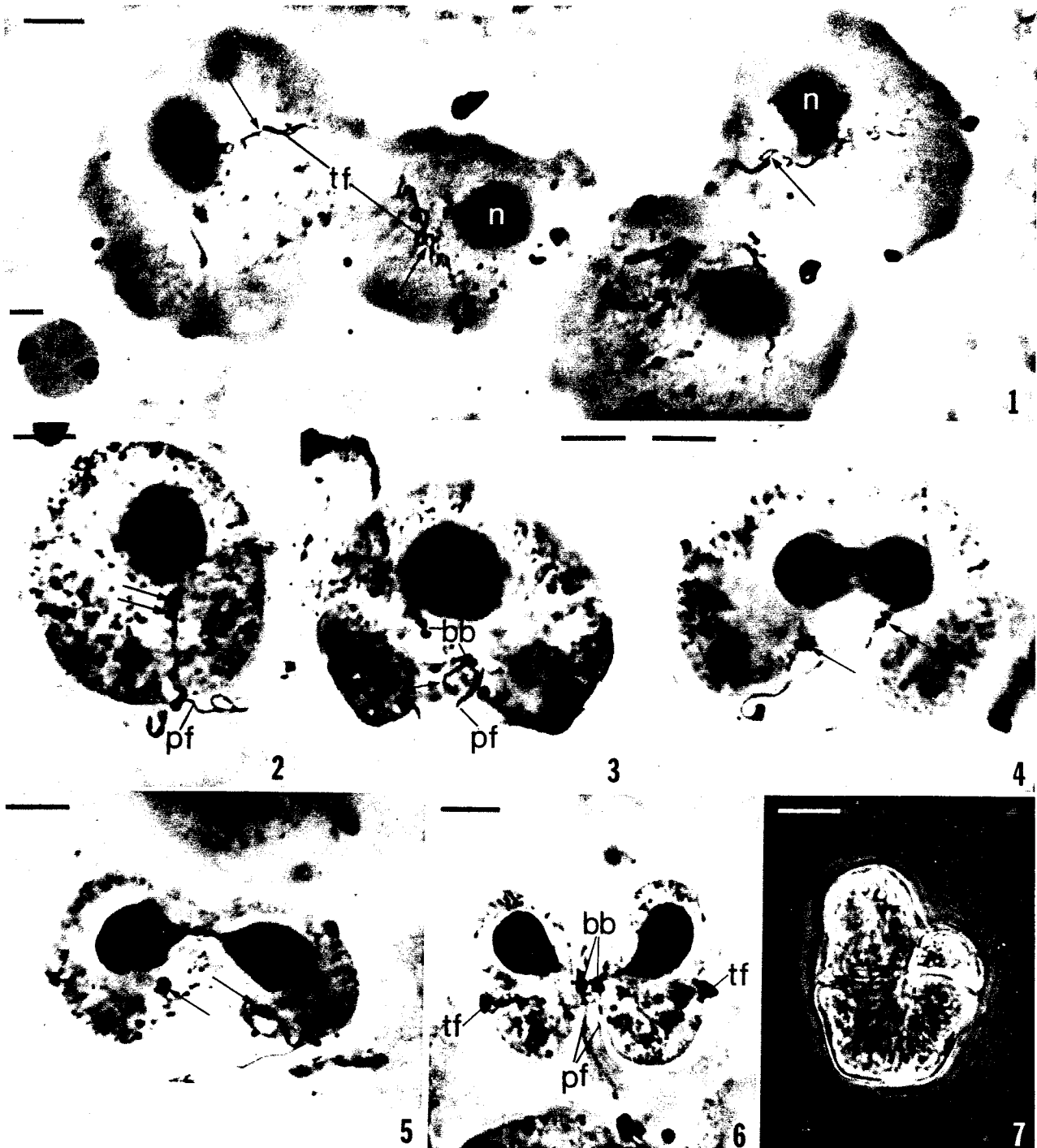
<sup>c</sup> Measurements represent the diameter of the spherical cyst nucleus.

The elevated period of division between  $T_{15}$  and  $T_{25}$  displayed two distinct pulses with each spanning 4–5 h and peaking with 15–20% of the population as dividing cells (Fig. 13). Cell density increased rapidly during this time and had almost doubled by the end of the first division pulse ( $T_{21}$ ). Apparently most individuals had completed one fission prior to the second division pulse. Cell counts continued to increase during the second pulse and reached ca.  $2100 \cdot \text{mL}^{-1}$  at  $T_{24}$ . That *Gyrodinium* numbers more than doubled from  $T_{15}$  to  $T_{24}$  suggests that at least some cells had divided twice. Following the second division peak, the number of *Gyrodinium* dropped to ca.  $1800 \cdot \text{mL}^{-1}$  thus showing a net two-fold increase over  $T_{15}$  cell density.

Near the end of the first division pulse, several pairs of *G. uncatenum* were observed loosely joined equatorially rather than apically as in late cell division. Cytological staining revealed such pairs as representing the earliest stage of gamete fusion. Gametes were smaller (ca. 0.25 of the cell volume of  $T_{15}$  cells) and rather variable in size but otherwise indistinguishable from pre-division trophic individuals (see Table 1). Since vegetative organisms and gametes had very similar morphologies, only those cells which had begun fusion could be unequivocally identified as gametes. Therefore, measurements for gametes presented in Table 1 were taken from cells in very early stages of fusion. Most coupled gametes

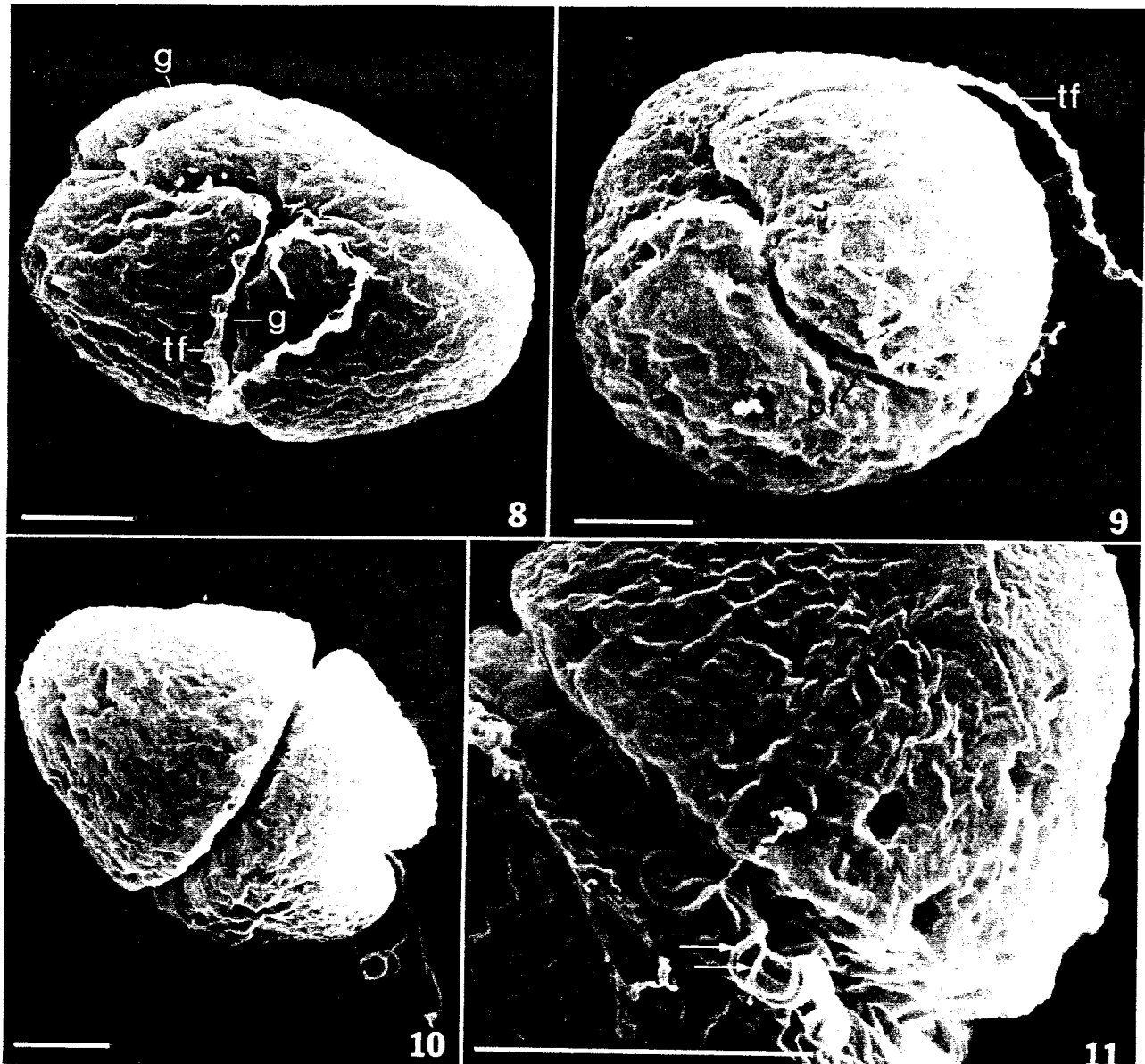
were of relatively equal size (Fig. 6), but a few were anisogamous. Anisogamous pairs were particularly abundant in old laboratory cultures and occasionally observed in field samples (Fig. 7). Paired gametes were typically oriented as mirror images, but their longitudinal axes were occasionally skewed to perpendicular as reported for *Ptychodiscus brevis* (Walker 1982). Asymmetric configurations were common in cultures, and gamete pairs were observed where the epicone of one cell was adjacent to the partner's hypocone. Misaligned gametes of *Woloszynskia apiculata* are reported to become reoriented prior to fusion (von Stosch 1973). The same appears true for *G. uncatenum* since gametes of later fusion stages were always aligned as mirror images.

Following the first division pulse, fusing gametes became progressively more numerous and peaked at ca. 22% of total motile cells mid-way through the second division pulse (Fig. 13). The nearly simultaneous rise in second division cells and coupled gametes suggests that gametes are fully differentiated and capable of fusing immediately after the second of two sequential divisions. Fusing gametes were abundant for ca. 7 h ( $T_{22}$ – $T_{29}$ ) but persisted in very low numbers until  $T_{86}$ . Cytological staining permitted close examination of gamete fusion, and the morphological events leading to planozygote formation are illustrated in Figure 14. The initial union of gametes occurred midventrally at the location of



NOTE: scales = 10  $\mu\text{m}$  for figures and 5  $\mu\text{m}$  for figure insets.

FIGS. 1-7. *Gyrodinium uncatenum*. FIG. 1. Four Protargol silver stained vegetative cells. Each organism has a single transverse flagellum (tf) with striated strand (arrows) and a deeply staining nucleus (n). Inset: Protargol stained trophic nucleus showing the typical complement of two nucleoli. FIG. 2. Protargol stained trophic cell showing the single posterior flagellum (pf) and the two flagellar bases (arrows). FIG. 3. An early stage in asexual division with both sets of paired basal bodies (bb) in focus. Also evident is the parental posterior flagellum (pf) and a nascent transverse flagellum (arrow); Protargol stain. FIG. 4. A mid-asexual division stage showing an increased separation between the two sets of basal bodies (arrows); Protargol stain. FIG. 5. Karyokinesis is nearly completed in this late division specimen and the basal bodies (arrows) of the two daughter cells are widely spaced. FIG. 6. A very early stage of gamete fusion. Gametes are of equal size and attached mid-ventrally at the location of the basal bodies (bb). The transverse flagellum (tf) and posterior flagellum (pf) of each cell are also discernible; Protargol stain. FIG. 7. A pair of anisogamous *G. uncatenum* gametes isolated from Parish Creek. Phase contrast of living material.



FIGS. 8–11. *Gyrodinium uncatenum*. FIG. 8. Scanning electron micrograph of a trophic cell showing the typical body shape and the transverse flagellum (tf) displaced from the girdle (g) during fixation. FIG. 9. Posterior view of a vegetative cell with the posterior flagellum (pf) emerging from deep in the sulcus; transverse flagellum (tf). FIG. 10. A recently excysted planomeiocyte with two posterior flagella. The two transverse flagella were lost during specimen preparation. FIG. 11. High magnification of the two transverse flagella (arrows) of a planomeiocyte.

the flagellar bases (Figs. 6 and 14a). From the onset, basal bodies of fusing gametes were in close proximity, in contrast to the “figure-8” stage of cell division (Fig. 12f). The nuclei of early fusers were elongated toward and linked to the basal bodies by an argentophyllic matrix. At this stage, the connection between the two gametes was similar to the fertilization tube of *Peridinium cinctum* f. *ovoplanum* (Spector et al. 1981); however, karyogamy occurred much later as reported for several other dinoflagellate species (von Stosch 1973, Pfeister 1976, 1977, Walker and Steidinger 1979). As plasmogamy proceeded, the two longitudinal flagella became aligned

and in living specimens were seen to function in unison. Simultaneously, the transverse flagellum of one gamete migrated out of the girdle and eventually lay adjacent to the transverse flagellum of the other gamete (Figs. 14b–d and 15–17). Upon completion of cytoplasmic fusion, forming zygotes possessed two trailing flagella, two transverse flagella beating together within the single girdle and two nuclei (Figs. 14d and 18). Planozygote formation was finalized by the fusion of the two nuclei to produce a nucleus usually containing 4 or 5 (range 3–7) nucleoli (Figs. 14e–f, 18 inset, 19 and 20).

Planozygote occurrence lagged behind and closely

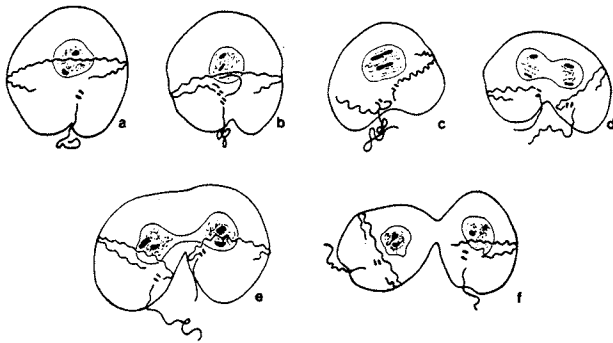


FIG. 12a-f. Semi-diagrammatic camera lucida drawings of asexual division in *Gyrodinium uncatenum* as revealed by Protargol silver staining.

paralleled the increasing abundance of fusing gamete pairs (Fig. 13). Between  $T_{23}$  and  $T_{40}$  the percentage of cells as planozygotes increased rapidly before leveling out to 80–85% of the population. The narrow gap between increasing percentages of paired gametes and planozygotes indicates that plasmogamy occurred quickly and required ca. 1 h for completion. Furthermore, the failure of total cell numbers to increase dramatically during the second division pulse is explained by the swift coupling and transformation of gametes into planozygotes. Since fusing gametes and planozygotes represent developmental stages endowed with two nuclear complements, the number of genomes present in samples

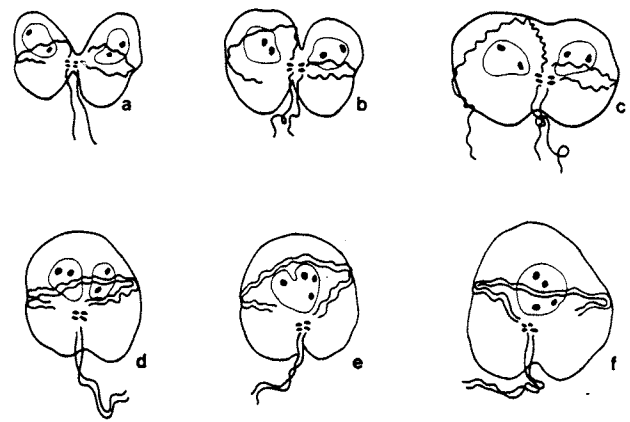


Fig. 14a-f. Semi-diagrammatic camera lucida illustrations of gamete fusion in Protargol stained *Gyrodinium uncatenum*.

can be obtained by appropriately adjusting total cell count; i.e. no. of genomes = no. of cells  $\cdot$  mL $^{-1}$  + (no. of cells  $\cdot$  mL $^{-1}$ )  $\cdot$  (% fusing gametes + % planozygotes). Manipulating the data accordingly produces the values shown in parentheses on Figure 13. Thus, between  $T_{15}$  and  $T_{40}$ , the total number of genomes showed a 3.6-fold increase from 930 to 3300  $\cdot$  mL $^{-1}$ . This nearly four-fold increase in genomes supports the concept that gamete differentiation requires two successive divisions. While other explanations are possible (viz. some cells dividing once with others dividing 2, 3, or more times), the former is consistent with the double peak in division frequency and with gametes having about one-fourth the cell volume of pre-division ( $T_{15}$ ) cells.

Planozygotes averaged  $43 \times 38 \mu\text{m}$  shortly after formation ( $T_{35}$ ), persisted without significant growth for several days and then encysted. Cysts were first evident at  $T_{51}$ , but accumulated slowly at first and only comprised ca. 1% of the population at  $T_{80}$ . Encystment proceeded more rapidly thereafter, with the population consisting almost entirely of hypozygotes at  $T_{100}$ . Planozygote maturation prior to encystment required 60–80 h. However, the timing of this period is rather variable and dependent upon environmental conditions (Anderson, unpublished observations). The actual encystment process appears more conservative and, once initiated, required slightly under 24 h for completion.

As discussed in Tyler et al. (1982), encysting planozygotes become less motile, lose much of their pigmentation, and round up. Eventually, the flagella are lost, clear storage granules form, and red pigment granules develop. Newly formed cysts from the isolated bloom population averaged  $48 \times 39 \mu\text{m}$ , possessed a spherical nucleus with four (range 3–6) nucleoli and three cyst walls (Table 1, Figs. 21, 22). The outer wall was loosely attached to the cyst and often removed by procedures used in cleaning and collecting cysts from sediments. By comparison, the middle wall was durable and rather rigid. The inner

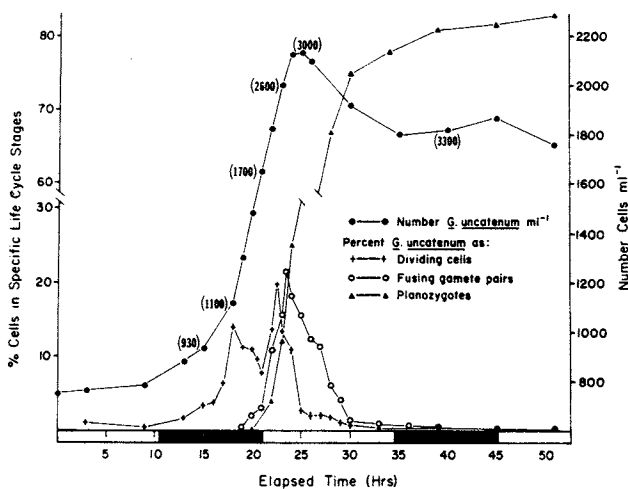
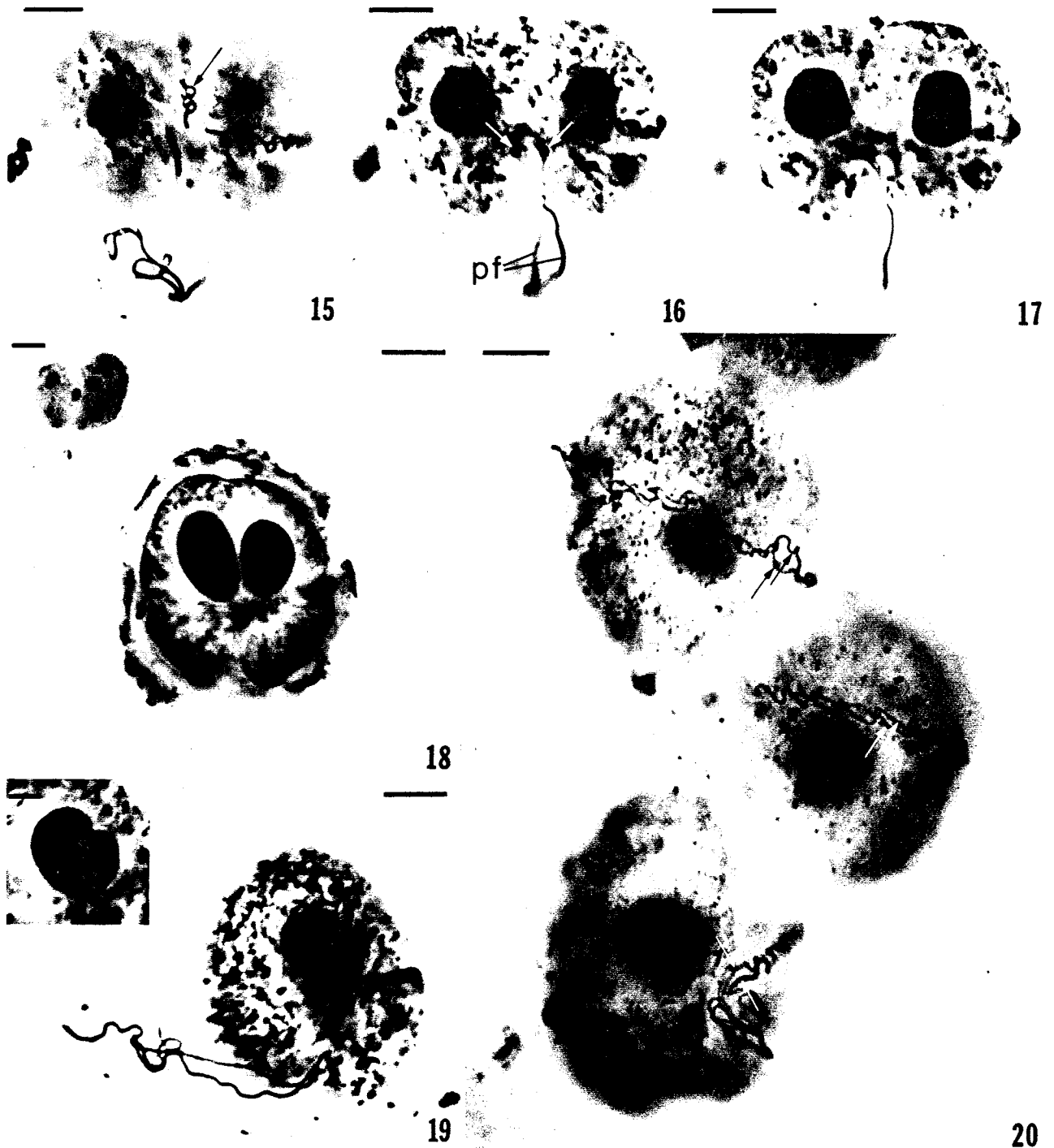
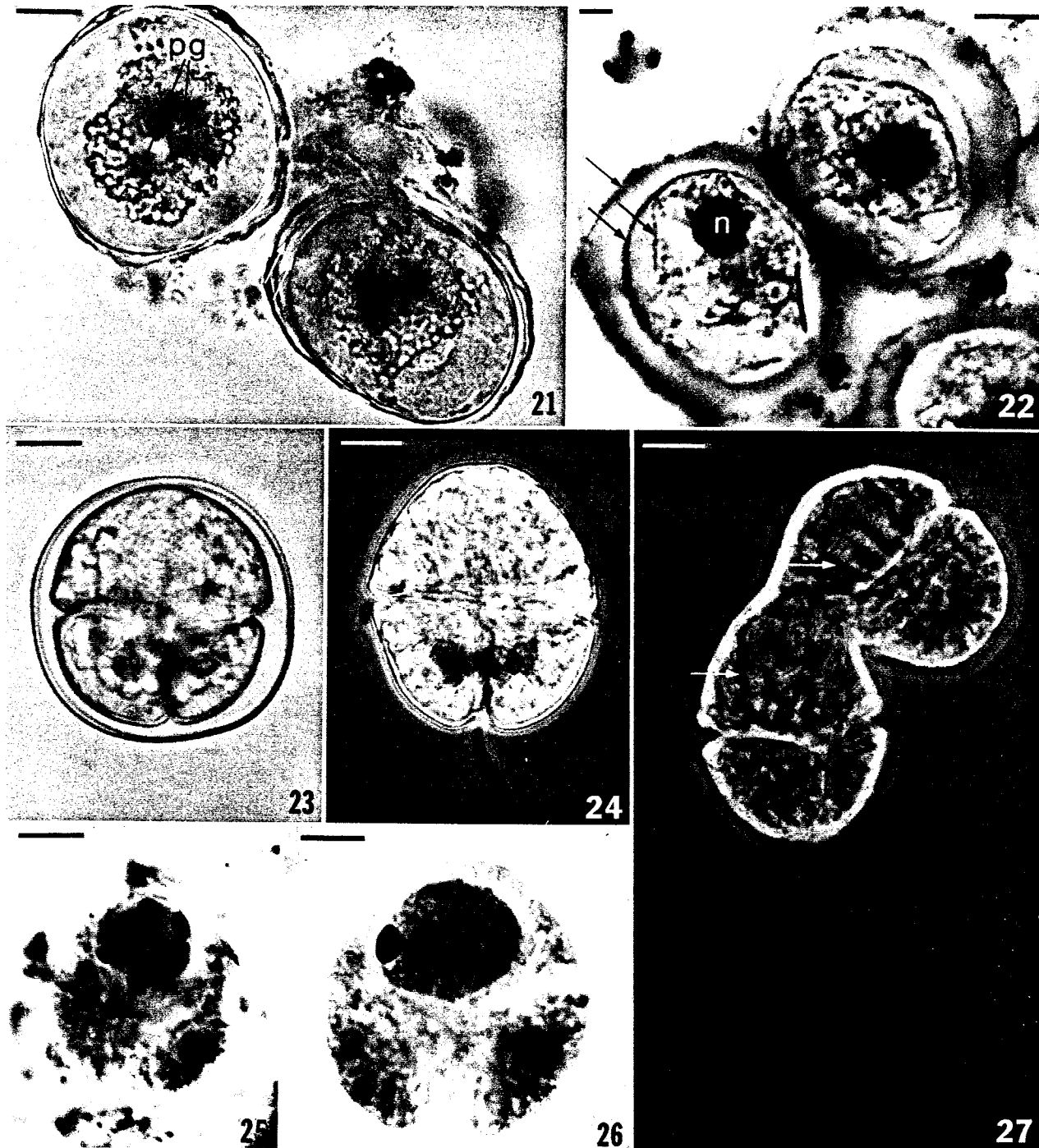


FIG. 13. The events associated with planozygote formation in the isolated *Gyrodinium uncatenum* population are graphically depicted. Abundances of major developmental stages are expressed as percentages of the population (left vertical axis). Percentages of dividing cells and fusing gametes were derived from alum hematoxylin preparations with  $n = 500$ . The frequency of planozygotes was obtained after Protargol staining with  $n = 100$ . Population densities, shown on the right vertical axis, represent single counts of 1-mL volumes. Numbers of genomes, computed as explained in the text, are shown in parentheses next to cell counts. Black areas indicate the interval between sunset and sunrise.



FIGS. 15-20. *Gyrodinium uncatenum*. FIGS. 15-17. This through-focal series of a mid-stage in gamete fusion shows the proximity of the two basal body pairs (white arrows) and the lack of a connection between the two nuclei. The posterior flagella (pf) are seen in close association. The transverse flagellum of one cell (arrow) is displaced from its girdle while the other remains firmly positioned; Protargol stain. FIG. 18. In this advanced stage of plasmogamy the two gametic nuclei remain distinct and apparently unjoined. Inset: A later stage in fusion where karyogamy has begun. Notice the presence of four nucleoli; Protargol stain. FIG. 19. The two trailing flagella are evident in this planozygote whose nucleus (inset) has almost completed fusion; Protargol stain. FIG. 20. A group of planozygotes where each cell bears two transverse flagella (arrows). The striated strand often stains lightly and is not visible in these specimens; Protargol stain.



FIGS. 21–27. *Gyrodinium uncatenum*. FIG. 21. Hypnozygotes with clear storage granules surrounding the dark orange-red pigment globules (pg). The three layered nature of the cyst wall is apparent at the white arrows; bright field, live specimens. FIG. 22. Protargol stained cysts showing the densely stained nucleus (n) and three cyst walls (arrows). Inset: A cyst nucleus containing four nucleoli. FIG. 23. A cell nearing excystment. Formation of the sulcus and the girdle are well advanced, red pigment globules are present posteriorly and numerous large clear granules are dispersed in the cytoplasm; bright field, live specimen. FIG. 24. Phase contrast of a newly excysted planomeiocyte with red pigment globules still present subequatorially; live specimen. FIG. 25. Protargol stain of a recently excysted planomeiocyte showing four nucleoli located peripherally within the nucleus. FIG. 26. A planomeiocyte nearing the first meiotic division. The enlarged nucleus contains a single nucleolus and large distinct chromosomes; Protargol stain. FIG. 27. Late in the first meiotic division. Notice the two posterior flagella of the lower daughter cell and the elongated chloroplasts (arrows); phase contrast, live specimen.

TABLE 2. Cell size and nuclear characteristics of developmental stages associated with excystment in *Gyrodinium uncatenum*.<sup>a</sup>

		Somatic			Nuclear			Nucleoli	
		L	W	n	L	W	n	No.	n
Cysts approaching excystment (T <sub>46</sub> )	$\bar{X}$	46.7	38.2	30	9.6 <sup>b</sup>		30	3.8	30
	Range	38.1-59.7	29.9-50.5		8.2-11.3			3-6	
	S	4.66	4.55		0.88			0.77	
	SE	0.85	0.83		0.16			0.14	
Recently excysted meiocyte (T <sub>50</sub> )	$\bar{X}$	35.0	28.2	30	12.4	11.4	30	3.8	30
	Range	29.9-42.2	24.7-35.0		10.3-17.5	9.3-14.2		2-6	
	S	3.31	2.60		1.46	1.09		0.95	
	SE	0.60	0.47		0.27	0.20		0.17	
Pre-division meiocyte (T <sub>80</sub> )	$\bar{X}$	34.6	31.0	30	17.0	14.5	30	1.4	27
	Range	30.9-40.2	23.7-36.0		13.4-20.6	12.4-17.5		1-3	
	S	2.44	2.98		2.16	1.24		0.70	
	SE	0.45	0.54		0.39	0.23		0.13	

<sup>a</sup> All observations are of Protargol silver stained specimens.

<sup>b</sup> Measurements represent the diameter of the spherical cyst nucleus.

wall, which may represent the cell membrane(s), was flexible and often crenulated after staining and dehydration.

**Excystment and planomeiocyte division.** Cysts collected from sediment in late winter readily excysted upon exposure to temperatures above 15°C. Aspects of cyst and meiocyte development were examined by stimulating freshly collected cysts to germinate. These cysts were morphologically consistent with those formed in the isolated bloom population. Pigment accumulations, storage granules, nucleoli number, and nuclear and somatic size were comparable for the two cyst populations (compare Tables 1 and 2). Many of the overwintered cysts lacked an outer wall which was presumably lost during sample preparation.

The earliest indication of excystment was visible 30-35 h after cysts were transferred to 20°C. Morphological changes included a widening of the gap between the middle and inner cyst walls and slight indentations at the developing sulcus and girdle. These features became more pronounced as ex-

cystment progressed and flagella were elaborated. Just prior to excystment, germinating cells still contained red pigment globules and numerous clear storage granules (Fig. 23). With development of flagella, zygotes became active within the cyst and eventually exited through ruptures in the middle and, when present, outer cyst walls.

Excystment began at ca. T<sub>45</sub>, where T<sub>0</sub> = time of transfer to 20°C, and proceeded steadily until ca. 95% of the cells were free swimming at T<sub>70</sub>. After excysting, *G. uncatenum* had few storage granules and little cytoplasmic coloration, but red pigment globules generally persisted in a subequatorial position (Fig. 24). The nuclei of these organisms were spherical to ovoid, slightly larger than those of cysts and usually contained four (range 2-6) nucleoli (Table 2, Fig. 25). Recently excysted *G. uncatenum* are presumably meiocytes, since planozygotes did not divide prior to encystment and only one cell emerged from the cyst. In addition, the persistence of four nucleoli from planozygote formation through cyst germination argues against the existence of nuclear reorganization during encystment. Like planozygotes, the meiocytes of *G. uncatenum* had two posterior and two transverse flagella (Figs. 10, 11), and

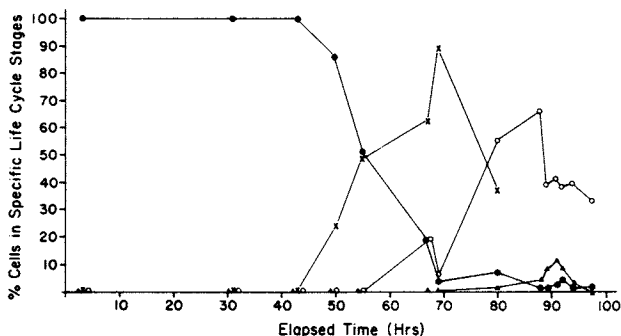


FIG. 28. Abundance of major developmental stages accompanying excystment in *Gyrodinium uncatenum*. Planomeiocytes with "Knauel"-stage nuclei (○) were determined from protargol stained samples (n = 30). The percentages of *Gyrodinium* as hypnozygotes (●), dividing cells (▲) and total planomeiocytes were determined from fixed unstained samples (n = 100). Planomeiocytes without "Knauel"-stage nuclei (×) were computed by subtraction.

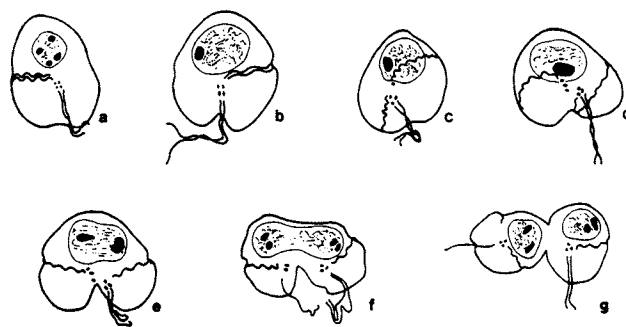


FIG. 29a-g. Semi-diagrammatic camera lucida illustrations of the sequential stages in planomeiocyte development from excystment through the first cell division: (a) recently excysted planomeiocyte; (b) "Knauel"-stage; (c-g) first meiotic division.

following the recommendation of von Stosch (1973) would be appropriately termed planomeiocytes. In the 24–48 h following excystment, planomeiocytes lost their red pigment globules, developed large chloroplasts characteristic of *G. uncatenum*, and took on an even reddish-brown coloration. During this time, the nucleus dramatically increased in size and eventually occupied much of the anterior portion of the cell (Fig. 26, Table 2). Enlarged nuclei possessed only one nucleolus and large, well defined chromosomes which were often paired. This condition represents the “Knauelstadium” described by Borgert (1910) and later associated with nuclear cyclosis and postzygotene phase of meiosis (Skoczylas 1958, von Stosch 1964, 1972). *Gyrodinium* with “Knauel”-stage nuclei appeared about 24 h after the start of excystment (Fig. 28). Cells whose nuclei contained a single nucleolus soon comprised ca. 65% of the population and then decreased in abundance with the onset of meiosis. Dividing cells were present in low numbers ca. 15 h after the development of “Knauel”-stage nuclei and reached a peak frequency of 11% at T<sub>91</sub>; thereafter, dividing organisms remained in low numbers.

The first and second meiotic divisions in *G. uncatenum* were not closely associated in time, and only cells in first division stages were encountered in stained preparations. Figure 29 illustrates the development of the planomeiocyte through the first meiotic division, as revealed by Protargol silver staining. Following nuclear reorganization, cell division began with one of the transverse flagella separating from the remaining three flagellar bases (Fig. 29c). Two new argentophyllic granules then formed in association with the isolated flagellar base, which resulted in a total of six granules arranged in two sets of three (Fig. 29d). The set of three “parental” flagella and basal bodies are destined for one daughter cell, while the other receives one “parental” transverse flagellum and differentiates a new posterior flagellum (Figs. 27, 29e–g). In late division stages, the cell with two flagella contained variably two or three argentophyllic granules, but only two bases appeared to persist after cytokinesis. Whether the triflagellated daughter resorbs one of its posterior flagella or returns to the vegetative configuration at the second division is unknown. As flagella were being redistributed, the already enlarged nucleus elongated and the single nucleolus usually divided twice, thus furnishing each daughter cell with the normal complement of two nucleoli.

The Protargol silver staining technique is routinely used in the study of ciliated protozoa, but is infrequently applied to flagellates. The procedure is somewhat tedious but simultaneously reveals nuclear and cortical structures. In the present study, Protargol staining proved useful in elucidating cytological aspects of sexuality in *G. uncatenum* and provided valuable markers for the identification and

enumeration of sexual stages in experimental and field populations.

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