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Comparative Morphology of the Euglenid Pellicle. I. Patterns of Strips and Pores

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ABSTRACT. In anticipation that improved knowledge of euglenid morphology will provide robust apomorphy-based definitions for clades, transmission and scanning electron microscopy were used to reveal novel morphological patterns associated with the euglenid pellicle. In some taxa, the number of pellicle strips around the cell periphery reduces as discrete whorls at the anterior and posterior ends of the cell. The number of whorls at either end varies between selected euglenid taxa but is invariant within a taxon. The pattern of strip reduction associated with these whorls is shown to have at least three evolutionarily linked states: exponential, pseudoexponential, and linear. Two general equations describe these states near the posterior end of euglenid cells. Exponential patterns of strip reduction near the anterior end are described by a third equation. In addition, several euglenid taxa were found to possess conspicuous pellicle pores. These pores are arranged in discrete rows that follow the articulation zones between adjacent strips. The number of strips between rows of pores varies between taxa and displays a series of consecutive character states that differ by a power of two. The patterns of pores may not only have phylogenetical and taxonomical value but may provide morphological markers for following strip maturation during cytoskeletal reproduction.

Key Words. Canal, character series, *Distigma, Euglena,* evolution, *Lepocinclis,* muciferous body, strip reduction.

THE pellicle of euglenids is a complex cell region comprised of the plasma membrane, supportive proteinaceous strips, subtending microtubules, and tubular cisternae of endoplasmic reticulum (Sommer 1965). The most conspicuous components of the pellicle are the parallel strips, which articulate along their lateral borders and are composed primarily of proteins known as articulins (Bouck and Ngo 1996; Dubreuil and Bouck 1985; Marrs and Bouck 1992). The characteristics of different pellicles, particularly the morphology and patterns of strips, appear to be intimately associated with different modes of nutrition and locomotion.

Although it is the defining apomorphy of the Euglenida, the pellicle has only rarely been systematically examined in detail (e.g. Angeler, Müllner, and Schagerl 1999; Buetow 1968; Cann 1986; Conforti and Tell 1989; Dragos, Péterfi, and Popescu 1997; Leedale and Hibberd 1974). The general objectives of both this paper and a forthcoming paper dealing with the diversity of strip substructure are (1) to outline macroevolutionary patterns of characters associated with the euglenid pellicle in order (2) to facilitate an accurate interpretation and classification of euglenid phylogeny. These morphological studies will lay down the groundwork for a larger contribution consisting of a molecular phylogeny of many taxa and the phylogenetic mapping of the morphological character states.

Here we describe novel patterns of strips and pores present on the cell surface of selected taxa with helical pellicles. These patterns are a consequence of the way strips terminate near the anterior and posterior end of the cell and the distribution of muciferous bodies beneath the pellicle. Different taxa consistently possess discrete pellicular patterns and these morphological data will be coupled with a maturing small subunit (SSU) rDNA database (e.g. BSL and MAF, unpubl. data; Linton et al. 1999; Linton et al. in press; Preisfeld et al. 2000). These combined data are expected to provide robust apomorphy-based definitions for important euglenid clades.

A subclade of euglenids (e.g. *Euglena, Phacus, Peranema, Distigma,* and *Colacium*) can be defined apomorphically by a pellicle with many strips (greater than 14) that are arranged helically (Linton et al. 1999; Montegut-Felkner and Triemer 1997; Triemer and Farmer 1991). These helical pellicles commonly permit the cell to distort its shape via the sliding strip model and produce wriggling movements termed ''euglenoid movement'' (Gallo and Shrével 1982; Petersen-Mahrt 1997; Suzaki and Williamson 1985). Euglenids with a helical pellicle also possess a complex flagellar opening comprised of an invaginated canal and reservoir (a posterior swelling of the canal) located at the anterior end of the cell. In general, both microtubules and the proteinaceous strips of the cell cortex migrate through the canal opening and form the inner cytoskeletal lining of the canal. Within the canal, the proteinaceous strips disappear gradually near the junction between the canal and reservoir, an anatomical position that may be termed the ''transition zone'' (Miller and Miller 1978). The microtubules of the pellicle pass around the reservoir and are continuous with those of one of the three flagellar roots, which functions as an MTOC (Farmer and Triemer 1988; Willey and Wibel 1985).

A few workers have reported that transverse sections through the anterior end of euglenid cells show a consistent number of pellicle strips around the canal. For example, *Euglena acus* has 14 strips around the canal (Mignot 1965), *Cyclidiopsis acus* has 16 (Mignot, Brugerolle, and Bricheux 1987*), Astasia longa* has 18 (Sommer and Blum 1965), and *Colacium libellae* has 20 (Willey and Wibel 1985). These data appear to form a character series. The number of strips around the canal is often half the number around the periphery of the cell. Correspondingly, some strips terminate near the canal opening of selected euglenid taxa (Angeler 2000; Bourrelly, Couté, and Rino 1976; Buetow 1968; Conforti and Tell 1983, 1989; Kirk and Juniper 1964; Leedale 1964, 1967; Sommer and Blum 1965).

Strips have also been observed to decrease in number toward the posterior end (Angeler 2000; Buetow 1968; Conforti and Tell 1983, 1989; Dawson and Walne 1991; Groupé 1947; Guttman and Ziegler 1974; Kirk and Juniper 1964; Leedale 1964, 1967; Mikolajczyk 1975; Sommer and Blum 1965; Suzaki and Williamson 1986). Kirk and Juniper (1964), Leedale (1964), and others have suggested that pairs of strips fuse into a single strip near the posterior end, which results in strip bifurcations (syn. ramifications—Angeler 2000; Angeler, Müllner, and Schagerl 1999). However, Guttman and Ziegler (1974) argued that strip reductions at the anterior and posterior ends do not occur by fusion but by undertucking.

Mucus-releasing bodies have been observed in euglenids via the application of dilute vital stains (Arnott and Walne 1967; Gojdics 1953; Leedale 1967). The descriptors ''mucocyst,'' ''muciferous body,'' and ''pellicle pore'' have been used interchangeably in the literature to describe these sac-like structures (Arnott and Walne 1967; Hausmann 1978; Hausmann and Mignot 1977; Mignot 1966; Willey 1984). It is useful to anatomically discriminate between these three descriptors. Mucocysts refer to large ejectile bodies that contain a highly organized structure composed of carbohydrate. These structures are rap-

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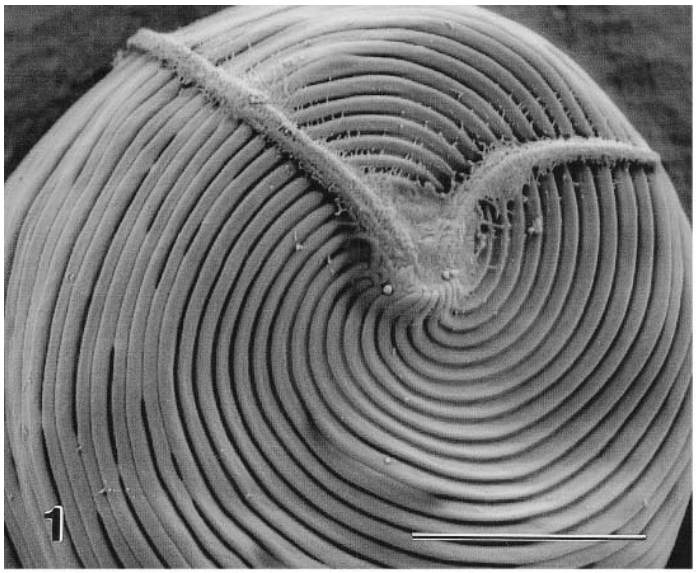
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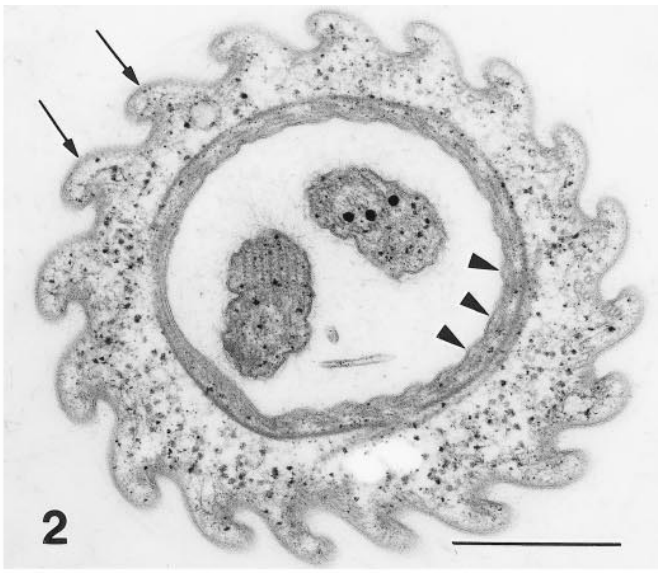
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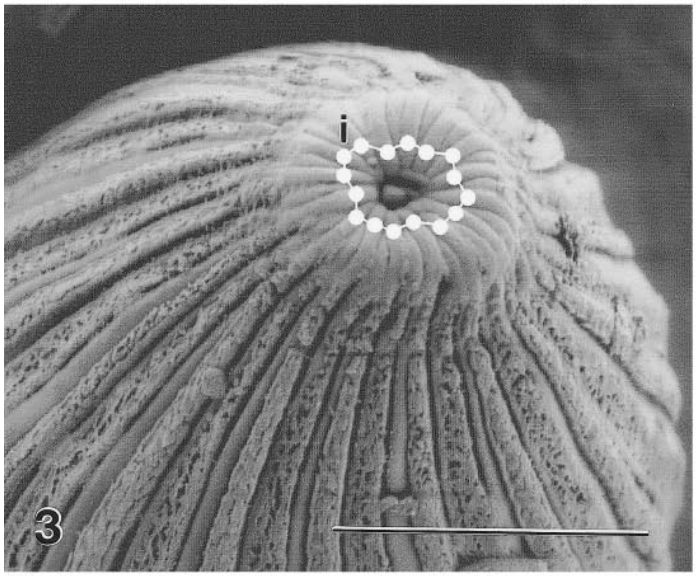
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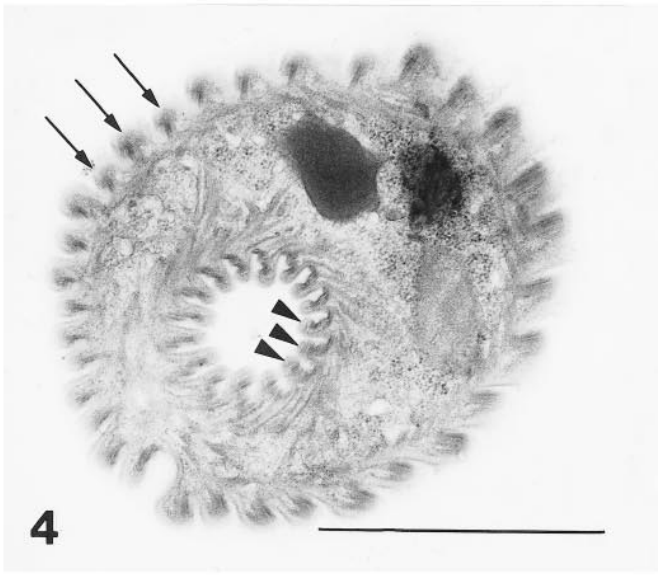
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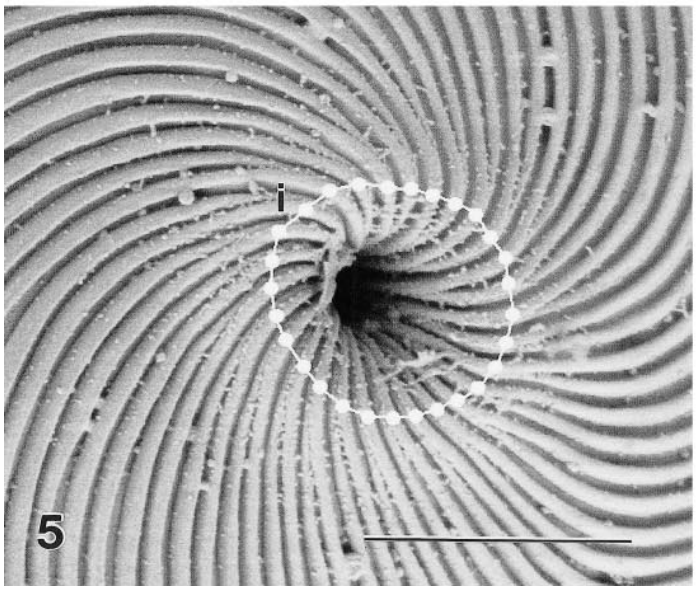
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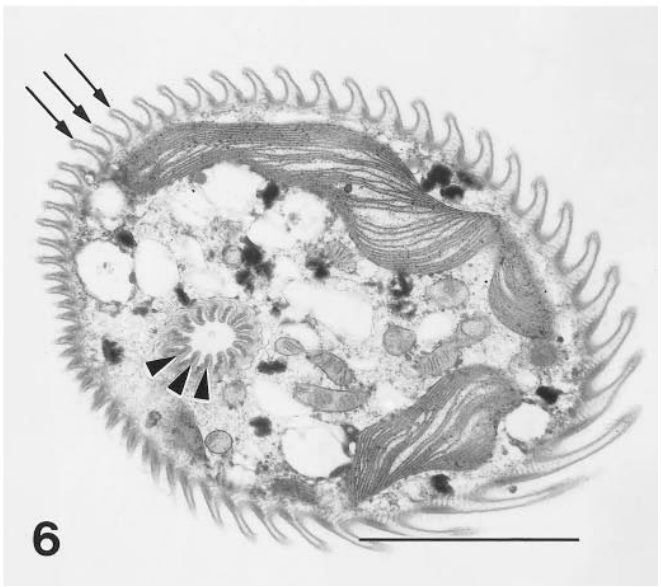
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idly ejected as long tubes supported by a lattice framework (Hilenski and Walne 1983; Mignot and Hovasse 1973). Mucocysts tend to be present in plesiomorphically colorless euglenids such as *Entosiphon* and *Peranema* (Hilenski and Walne 1983; Mignot and Hovasse 1973).

In this paper, ''muciferous body'' refers to a subpellicular compartment containing a water-soluble mucupolysaccharide that is either fibrous or amorphous in structure (Hausmann and Mignot 1977; Leedale 1967; Mignot 1966). Muciferous bodies tend to occur in phototrophic euglenids. Accordingly, mucocyst-like organelles were probably the precursors to muciferous bodies. ''Pellicle pore'' refers to the small opening that is positioned within the articulation zones of adjacent strips through which amorphous material in each muciferous body may be discharged. As a source of possible confusion, some authors have used ''muciferous body'' to refer to the tubular cisternae of endoplasmic reticulum (ER) associated with each pellicle strip (e.g. Buetow 1968; Leedale 1967; Leedale, Meeuse, and Pringsheim 1965; Dragos, Péterfi, and Popescu 1997). These cisternae of ER and the mucus-secreting bodies examined in this paper are clearly separate compartments containing different substances (e.g. Arnott and Walne 1967; Murray 1981; Triemer 1980). The functions of muciferous bodies may include but are not limited to stalk formation in *Colacium,* lorica formation in *Trachelomonas* and *Strombomonas,* cyst formation, and a lubricant for euglenoid movement (Hilenski and Walne 1983; Leedale 1967; Leedale, Meeuse, and Pringsheim 1965; Olli 1996; Rosowski and Willey 1977; Triemer 1980).

METHODS AND MATERIALS

Culture conditions. All cultures were maintained in an incubator at 20 °C and programmed for a 12 h light–12 h dark cycle. Cultures of *Euglena gracilis* (UTEX 753), *Euglena mutabilis* (SAG 1224–9a), *Lepocinclis buetschlii* (UTEX LB 523), *Distigma proteus* (UTEX LB 508), *Euglena cantabrica* (UTEX LB 1320), *Euglena myxocylindracea* (UTEX LB 1989), and *Euglena terricola* (UTEX LB 1310) were purchased from either the Culture Collection of Algae at the University of Texas at Austin (UTEX) or Sammlung von Algenkulturen Göttingen (SAG). *Euglena gracilis* and *E. myxocylindracea* were grown in *Euglena gracilis* medium (EG, Greenblatt and Schiff, 1959). *Euglena mutabilis* was grown in equal parts of EG medium and soil-water extract. *Lepocinclis buetschlii, E. cantabrica,* and *E. terricola* were grown in soil-water medium with ammonium magnesium phosphate hexa-hydrate (0.1 g / 200 ml). *Distigma proteus* was maintained in soil-water medium with crushed barley. An undescribed species that conforms to the diagnosis of *Euglena* (Gojdics 1953) was isolated from a bloom in marine sediments on Sapelo Island, Georgia and is designated as *Euglena* sp.

Transmission electron microscopy. Cells were concentrated into Eppendorf tubes by slow centrifugation. Cells were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.2) at 4 °C for 1 h. Following primary fixation, the cells were washed in 0.1 M cacodylate buffer (pH = 7.2) for two changes of 15 min, each. Post-fixation was for 1 h in 1% O_SO_4 and cacodylate buffer (pH = 7.2) at 4 °C. The cells were washed with distilled water, dehydrated through a graded series of ethyl alcohols, and submerged in acetone for two changes of 20 min, each. The cells were infiltrated with acetone-resin mixtures and embedded in pure resin (EMS); samples were spun down at high speed into the tip of an embedding capsule. Blocks were polymerized at 60 °C and sectioned on a RMC MT-X ultramicrotome, post-stained with uranyl acetate and lead citrate, and viewed under a JEOL 100 CX II Transmission electron microscope.

Scanning electron microscopy. A small volume (~ 10 ml) of cells in liquid medium was transferred into a small Petri dish that contained a piece of filter paper, saturated with 4% O_SO_4, mounted on the inner surface of the lid. The lid was placed over the chamber and the cells were fixed by O_SO_4 vapors for 30 min. Four to five drops of 4% O_SO_4 were added directly into the liquid medium and the cells were fixed for another 30 min. The cells were transferred onto 8-μm polycarbonate membrane filters (Corning Separations Div., Acton, MA), dehydrated with a graded series of ethyl alcohol, and critical point dried with CO_2. Filters were mounted on stubs and sputter coated for 60 s (~ 153 Å) with gold. The cells were viewed under a LEO 982 Scanning electron microscope.

Replicate observations. Distinct patterns of pores and terminating strips were observed on the taxa listed above. In order to examine whether patterns were consistent within taxa, 30 different cells were scored for each pattern. The mode and range of variation were recorded for each pattern observed.

RESULTS

Patterns of pellicle strips. The arrangement of strips near the canal opening varied between selected taxa with helical pellicles. In *D. proteus,* 18 strips surrounded the periphery of the cell and migrated into the canal opening (Table 1 and Fig. 1, 2). Shortly after entering the canal, all 18 proteinaceous strips terminated leaving 18 sets of microtubules to form the canal cytoskeleton (Fig. 2). In *L. buetschlii,* 16 strips terminated before entering the canal opening (Fig. 3). A single strip that continued into the canal was positioned between two consecutively terminating strips. Therefore, 32 strips surrounded the periphery of the cell and 16 strips lined the canal (Table 1 and Fig. 4). The 16 strips that terminated before entering the canal formed a single discrete whorl of strip reduction (Fig. 3). Whorl ''*i*'' was defined as the first whorl near the canal opening on which the number of strips around the periphery (the maximum number of strips) is incompletely reduced. The number of strips was halved across whorl *i* making the pattern of strip reduction exponential (Table 1).

In some *Euglena* sp., 28 strips terminated just outside the

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Fig. **1**–**6**. Patterns of pellicle strips near the anterior end of euglenid cells. **1**. SEM of the anterior end of *Distigma proteus.* Notice the absence of strip reductions near the canal opening (Bar = 4 μm). **2**. A TEM transverse section through the canal of *Distigma proteus.* Notice 18 peripheral strips (arrows) and 18 sets of microtubules (arrowheads) that lack distinct strips surrounding the canal (Bar = 0.5 μm). **3**. SEM of *Lepocinclis buetschlii.* Dots mark 16 pellicle strips that terminate before entering the canal opening and form a whorl of strip reduction (*i*). To avoid obscuring strip termination events, the dots were positioned to the side of each termination point (Bar = 2 μm). **4**. A TEM transverse section through the canal of *Lepocinclis buetschlii.* Notice 32 peripheral strips (arrows) and 16 distinct strips surrounding the canal (arrowheads) (Bar = 3 μm). **5**. SEM of *Euglena* sp. Dots mark 28 pellicle strips that terminate before entering the canal opening and form the first whorl of strip reduction (*i*) at the anterior end of the cell. To avoid obscuring strip termination events, the dots were positioned to the side of each termination point (Bar = 4 μm). **6**. A TEM transverse section through the canal of *Euglena* sp. Note the 56 peripheral strips (arrows) and 14 distinct strips surrounding the canal (arrowheads) indicating an exponential pattern of strip reduction near the canal that consists of two separate whorls (*i* & *ii*) (Bar = 2 μm).


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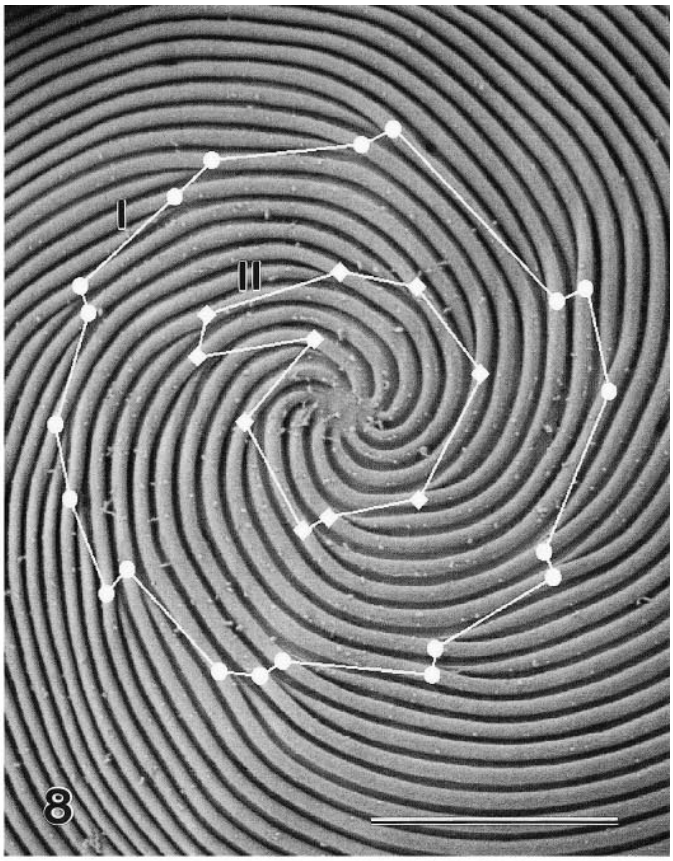
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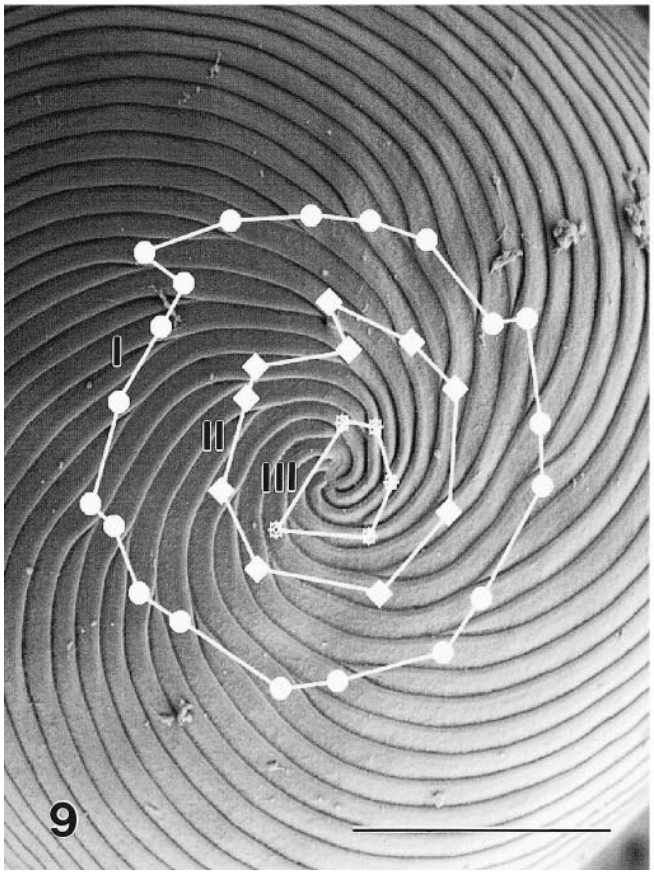
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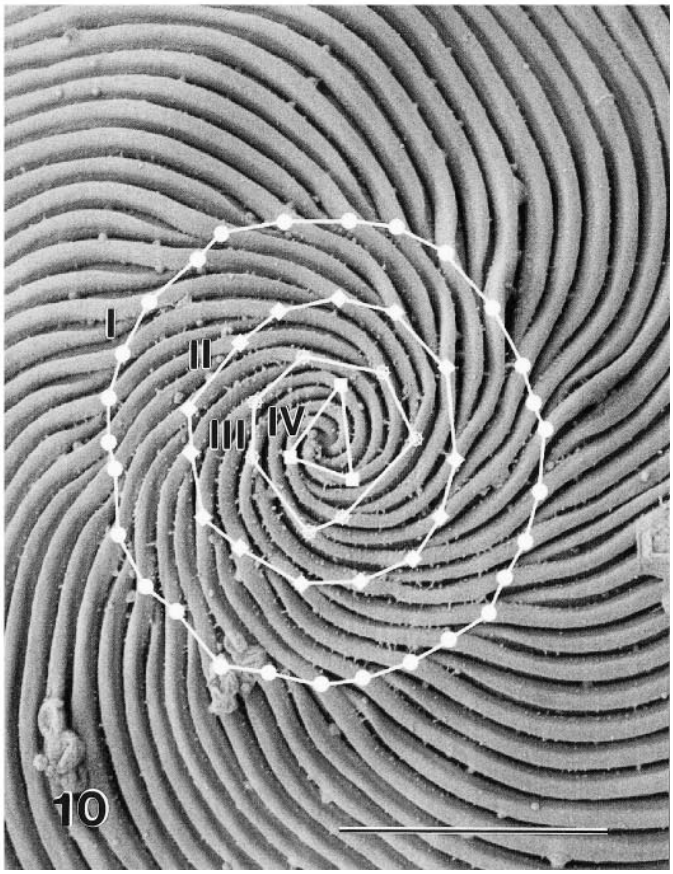
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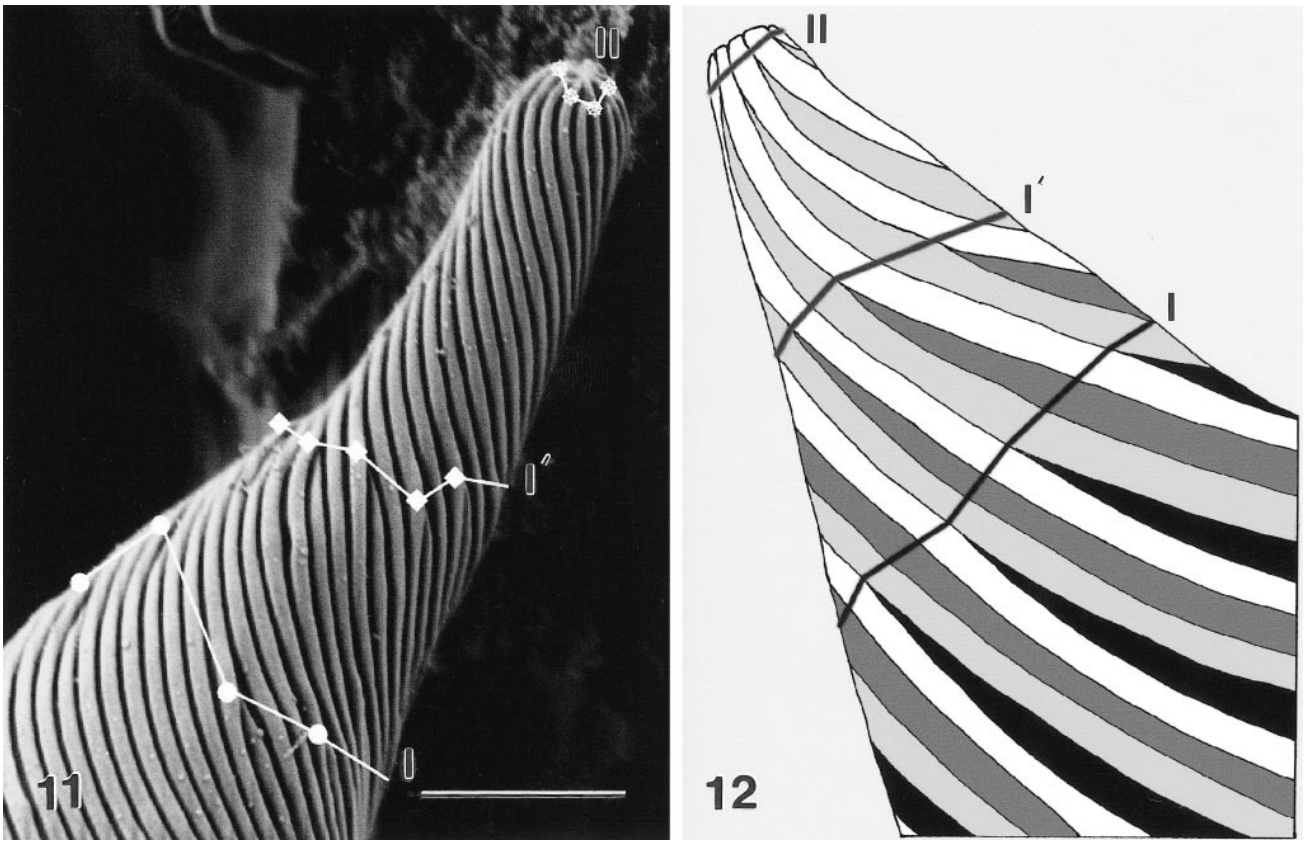

Fig. **11**–**12**. Linear pattern of strip reduction near the posterior end of *Euglena mutabilis.* **11**. SEM showing three whorls of strip reduction. Dots mark the 10 strips that terminate on the anterior (outer) whorl I. Diamonds mark the 10 strips that terminate on the middle whorl I′. Asterisks mark the 10 strips that terminate on the posterior (inner) whorl II. Ten strips converge at the posterior tip (Bar = 2 μm). **12**. A diagram illustrating the linear pattern of strip reduction. Three strips pass between consecutively terminating strips on anterior whorl I. Two strips pass between consecutively terminating strips on middle whorl I′. One strip passes between consecutively terminating strips on posterior whorl II.

canal opening, and like *L. buetschlii*, a single strip that continued into the canal was positioned between each pair of terminating strips (Fig. 5). Thus, whorl *i* was also present in *Euglena* sp., however, in this taxon, a second whorl of strip reduction occurred within the canal that was identified as whorl ''*ii*''. A transverse section anterior to the transition zone demonstrated 56 strips around the cell periphery and 14 strips around the canal (Fig. 6). The pattern of strip reduction was exponential as the number of strips was halved across whorls *i* and *ii* (Table 1). Other individuals in this taxon had 60 strips around the cell periphery that also reduced exponentially across two whorls (60 → 30 → 15) (data not shown).

The arrangement of strips near the posterior end of cells varied between taxa. In *D. proteus,* all 18 strips around the cell periphery met at the posterior tip (Fig. 7). By contrast, in *E. myxocylindracea,* 40 strips surrounded the cell periphery and 20 strips terminated before reaching the posterior tip. A single strip was positioned between consecutively terminating strips and continued toward the cell posterior; 20 strips passed through this whorl of strip reduction (Fig. 8). Whorl ''I'' was defined as the first whorl near the posterior end on which the number of strips around the periphery (the maximum number of strips) is incompletely reduced. In *E. myxocylindracea,* ten out of the 20 strips that passed through whorl I terminated before reaching the posterior tip and formed a second discrete whorl of strip reduction, whorl ''II'' (Fig. 8). On whorl II, the

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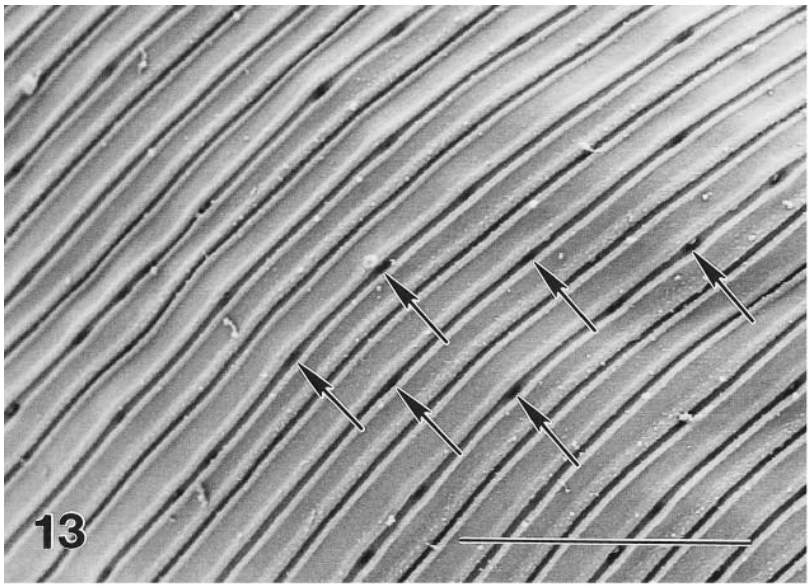
Fig. **7**–**10**. Patterns of terminating strips near the posterior end of euglenid cells. To avoid obscuring strip termination events, the markers were positioned to the side of each termination point. **7**. SEM of *Distigma proteus.* Notice that all 18 strips converge at the posterior tip (Bar = 3 μm). **8**. SEM of *Euglena myxocylindracea* showing an exponential pattern of strip reduction with two whorls. Dots mark the 20 strips that terminate before reaching the posterior tip and form whorl I. Diamonds mark 10 strips that terminate before reaching the posterior tip and form whorl II. Ten strips converge at the posterior tip (Bar = 2 μm). **9**. SEM of *Euglena gracilis* displaying an exponential pattern of strip reduction with three whorls. Dots mark the 20 terminating strips that form whorl I. Diamonds mark the 10 terminating strips that form middle whorl II. Asterisks mark the 5 terminating strips that form a third inner whorl III. Five strips converge at the posterior tip (Bar = 3 μm). **10**. SEM of *Euglena* sp. showing a pseudoexponential pattern of strip reduction with four whorls. Dots mark the 28 terminating strips that form whorl I. Diamonds mark the 14 terminating strips that form middle whorl II. Asterisks mark seven strips that terminate and form a second middle whorl III. The seven remaining strips cannot be halved; thus strips do not reduce exponentially across a final inner whorl IV. Consequently, three terminating strips (squares) form whorl IV and the four remaining strips converge at the posterior tip (Bar = 3 μm).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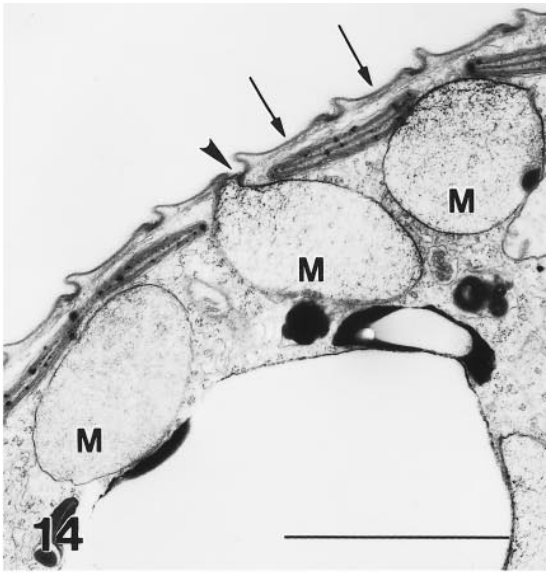
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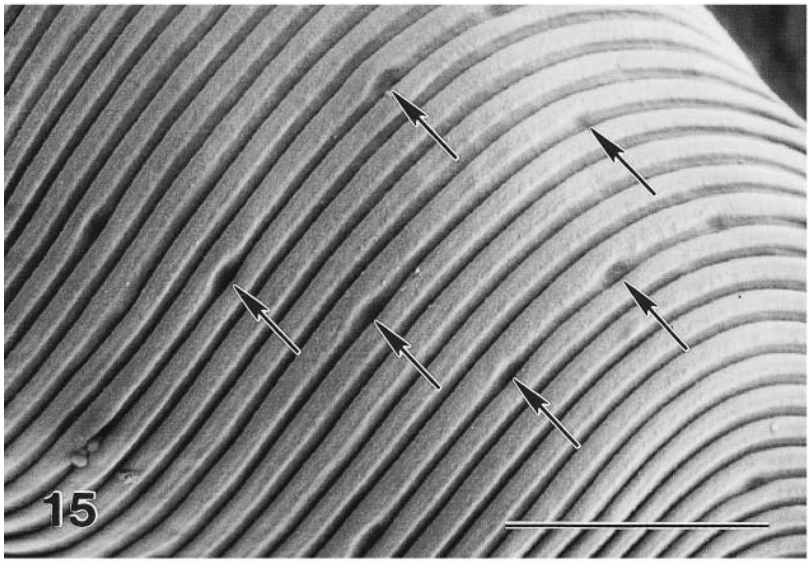
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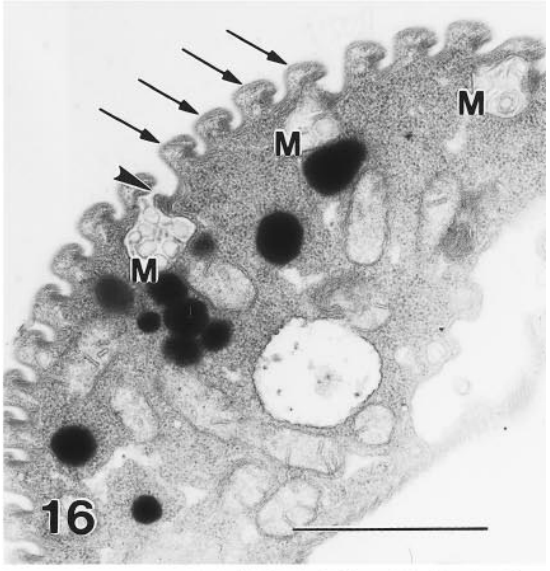
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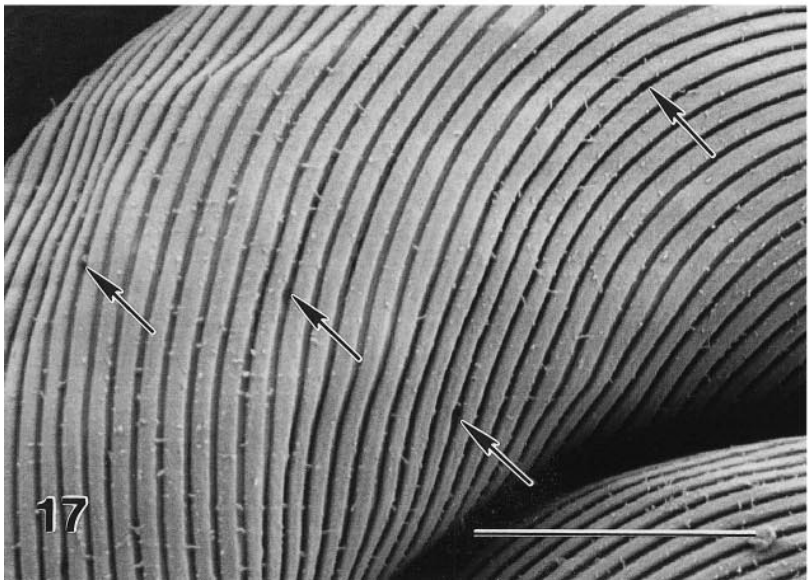
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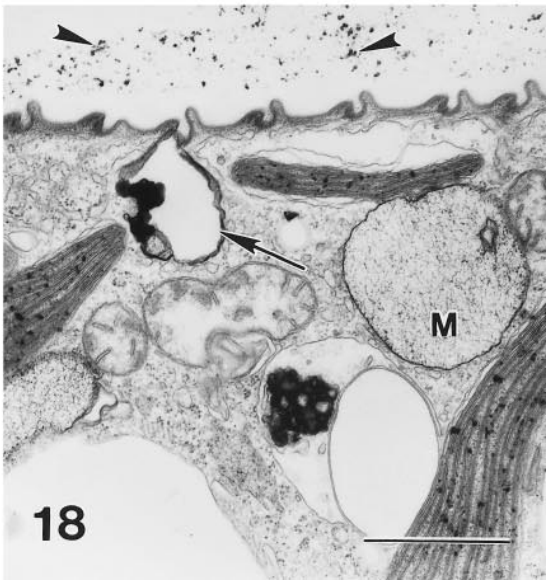
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Table **1**. The number of strips on the euglenid pellicle reduced on each whorl. (refer to Fig. 1–11). For each pair of numbers, the first refers to the number of strips entering a particular whorl and the second is the number of strips that continue through the whorl. ''P'' refers to the number of strips around the periphery; the range of variation is shown parenthetically to the mode. ''n'' refers to the frequency of the mode for P over the number of cells observed.

	Taxon					
Character	*Distigma proteus* (n = 30/30)	*Lepocinclis buetschlii*[a] (n = 30/30)	*Euglena* sp.[b] (n = 23/30)	*Euglena gracilis*[a,b] (n = 20/30)	*Euglena myxocylindracea* (n = 26/30)	*Euglena mutabilis*[c] (n = 23/30)
P	18	32	60(56, 60)	40(34–49)	40(40–48)	40(36–48)
Anterior whorls						
i	—	32–16	56–28	40–20	40–20	40–20
ii	—	—	28–14	—	—	—
Posterior whorls						
I	—	32–16	56–28	40–20	40–20	40–30
I′	—	—	—	—	—	30–20
II	—	16–8	28–14	20–10	20–10	20–10
III	—	—	14–7	10–5	—	—
IV	—	—	7–4[d]	—	—	—

[a] Exponential pattern of strip reduction at posterior end.
[b] Pseudoexponential pattern of strip reduction at posterior end.
[c] Linear pattern of strip reduction at posterior end.
[d] The whorl in which the exponential pattern breaks down.

ten strips that were positioned between consecutively terminating strips ultimately met at the posterior tip. The pattern of strip reduction was exponential (Table 1). Although the most common number of peripheral strips scored was 40 (the mode), four individuals possessed 42, 44, 46, and 48 strips, respectively (Table 1). These alternative states differed by two strips. Regardless of this variability, the pattern of strip reduction near the posterior end always formed two whorls of strips that reduced exponentially (e.g. 48 peripheral strips reduced to 24 across whorl I and 24 to 12 across whorl II–data not shown).

Euglena gracilis had a similar pattern of strip reduction. However, in this taxon a third whorl of strip reduction, whorl ''III'', was present near the posterior end of the cell. Forty strips were reduced to 20 across whorl I, 20 strips were reduced to 10 across whorl II, and 10 strips were reduced to five across whorl III (Table 1 and Fig. 9). Although the most common number of peripheral strips was 40, the number of strips ranged from 34 to 49 (Table 1). Regardless of this variability, the pattern of strip reduction near the posterior end always formed three whorls (e.g. 48 to 24, 24 to 12, and 12 to 6). The pattern of strip reduction in *E. gracilis* is exponential because the number of strips was halved across whorls I, II, and III (Table 1).

The pattern of strip reduction near the posterior end of *Euglena* sp. was slightly more complex than the patterns described above. There were four whorls of strip reduction, regardless of the number of peripheral strips. The number of peripheral strips in this taxon was either 56 (Fig. 10) or 60 (Table 1). In the same alternating manner of strip termination described for *E. gracilis,* the number of strips was halved across whorls I, II, and III (Table 1). However, because the number of strips around the periphery was not wholly divisible by 16 (the fourth halving event within an exponential pattern of decay, 2^4), the number of strips was asymmetrically reduced across whorl IV (Table 1). Seven strips entered whorl IV; three strips terminated and four strips continued through the whorl and met at the posterior tip. This slightly different pattern of strip reduction we define as pseudoexponential.

A third pattern of strip reduction near the posterior end was found in *E. mutabilis.* In this taxon, there were three discrete whorls of strip reduction, but the strips did not reduce exponentially across the whorls (Fig. 11). A linear pattern of strip reduction occurred as the same number of strips terminated on each whorl. This constant increment of terminating strips corresponded to the number of strips that met at the posterior tip, 10. Thus, 40 strips around the cell periphery were reduced to 30 by reduction of every fourth strip across whorl I (Fig. 11, 12). The 30 strips that passed through whorl I were further reduced every third strip to 20 across whorl ''I′'' (This peculiar notation is justified later). The 20 strips that passed through whorl I′ were reduced by every alternate strip to 10 across whorl II. The 10 strips that passed through whorl II met at the posterior tip. Although there was some variability in the number of strips around the cell periphery (Table 1), the linear pattern of strip reduction across three whorls was still observed in all individuals. For instance, when the peripheral strip number was 36, strips were reduced from 36 to 27 across whorl I, 27 to 18 across whorl I′, and 18 to 9 across whorl II (data not shown).

Patterns of pellicle pores. Although some taxa lacked conspicuous pellicle pores when viewed under the SEM (e.g. *D. proteus*), many others possessed pellicle pores between strips.

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Fig. **13**–**18**. Patterns of pellicular pores in the euglenid pellicle. **13**. SEM of *Euglena cantabrica* showing two strips between rows of pellicle pores (arrows) (Bar = 4 μm). **14**. Oblique TEM section through three adjacent muciferous bodies (M) showing two pellicular strips (arrows) between the muciferous bodies. The muciferous bodies are compartments below each pellicle pore (arrowhead) (Bar = 2 μm). **15**. SEM of *Euglena terricola* showing four strips between rows of pellicle pores (arrows) (Bar = 3 μm). **16**. Oblique TEM section through three adjacent muciferous bodies (M) on separate rows of pores (arrowhead). Four strips (arrows) reside between the muciferous bodies (Bar = 1 μm). **17**. SEM of *Euglena myxocylindracea* showing eight strips between rows of pellicle pores (arrows) (Bar = 4 μm). **18**. TEM of *Euglena cantabrica* showing the morphology of a muciferous body (M) and one that probably has expelled its contents (arrow). The material around the cell surface (arrowheads) is presumably the discharged mucus (Bar = 1 μm).

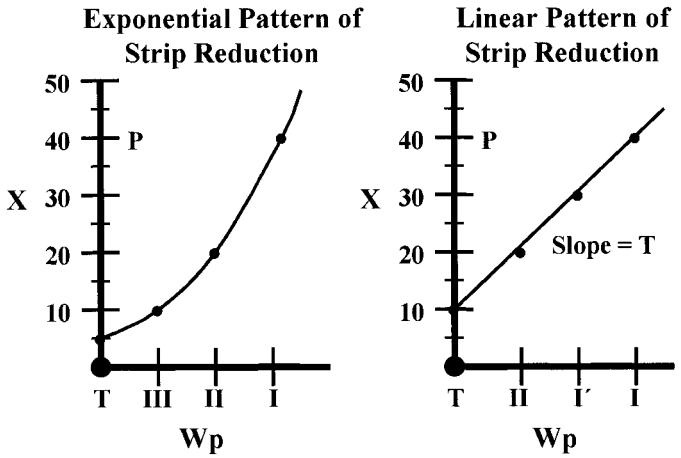

Fig. **19**. Graphical representations of exponential (*Euglena gracilis*) and linear (*Euglena mutabilis*) patterns of strip reduction on the euglenid pellicle. Symbols are defined in Table 2.

The abundance and distribution of these pores ranged from sparse and scattered (e.g. *E. gracilis* and *E. mutabilis*) to dense and organized. When many pellicle pores were present, they were arranged in rows that ran parallel to the strips. The number of strips between rows of pellicle pores was very consistent within taxa yet varied between taxa. *Euglena cantabrica* had two strips between rows of pellicle pores Table 3 and Fig. 13, 14). *Euglena terricola* usually had four strips between rows of pores (Table 3 and Fig. 15, 16). *Euglena myxocylindracea* usually had eight strips between rows of pores (Table 3 and Fig. 17).

In oblique sections, adjacent muciferous bodies are positioned between pellicle strips and below different rows of pores (Fig. 14, 16, 18). These data demonstrate that muciferous bodies open to the external environment via pellicle pores positioned within the articulation zones between strips. TEM data were also consistent with the SEM data, in that two strips separated rows of muciferous bodies in *E. cantabrica* (Fig. 14) and four strips separated rows of muciferous bodies in *E. terricola* (Fig. 16).

DISCUSSION

In many euglenids with helical pellicles, there is a reduction in strip number toward the anterior and posterior poles. We have examined strip reductions in a number of taxa and have identified variable characters that may be phylogenetically informative. Many of these characters are linked within discrete patterns that can be expressed mathematically. Before describing these general equations, however, it is first necessary to define the relevant characters (Table 2).

''C'' refers to the minimum number of strips that surround the canal, ''P'' is the maximum number of strips around the periphery of the cell, and ''T'' is the number of strips that meet at the posterior tip (syn. the posterior vortex). ''W_A'' refers to the number of whorls of strip reduction near the anterior end, where a lower case Roman numeral denotes each whorl (subscript ''$_A$'' = anterior). ''W_P'' refers to the number of whorls of strip reduction near the posterior end, where an uppercase Roman numeral denotes each whorl (subscript ''$_P$'' = posterior). ''X'' refers to the number of strips immediately preceding a whorl of strip reduction, where X_I denotes the number of strips preceding whorl I, X_{II} denotes the number of strips preceding whorl II, and so forth. ''S'' refers to the number of strips between two consecutive strips that terminate on a whorl, where

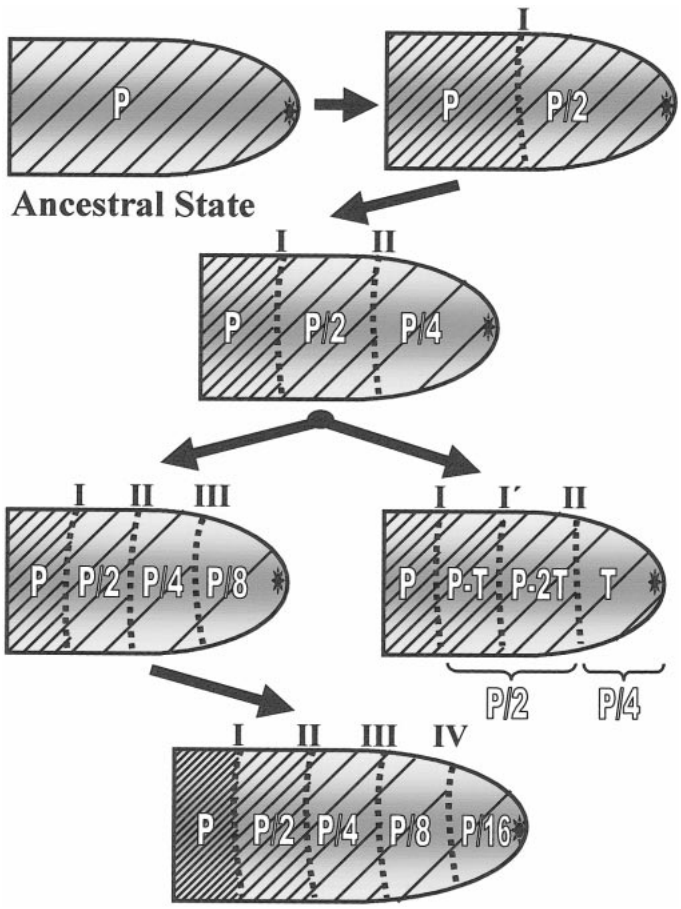

Fig. **20**. Flowchart illustrating the hypothetical evolutionary pathways that led to the patterns of posterior strip reduction. ''P'' is the number of strips around the cell periphery, and ''T'' is the number of strips that meet at the posterior tip. A pellicle in which all the peripheral strips meet at the posterior tip (P = T) is hypothesized to represent the ancestral state. The first whorl on which the number of strips is reduced is defined as whorl I. All other whorls within exponential and pseudoexponential patterns of strip reduction are tagged in reference to whorl I, so that the next whorl closer to the posterior end is labeled ''II'' and so forth. In exponential patterns, the number of strips is halved across every whorl. In linear patterns, the number of strips is reduced by a factor of T (a constant increment of strip reduction) across every whorl. The two most anterior whorls within a linear pattern of strip reduction are inferred to be homologous to whorl I within exponential patterns. Whorl I refers to the most anterior whorl and whorl I′ refers to the next inner whorl within linear patterns of strip reduction. The most posterior whorl in a linear pattern of three whorls is labeled ''II'' because it is inferred to be homologous to whorl II of exponential patterns of strip reduction.

S_I denotes the number of strips between two consecutively terminating strips on whorl I, S_{II} denotes the number of strips between two consecutively terminating strips on whorl II, and so forth.

A whorl of strip reduction can be recognized whenever X is incompletely reduced. By definition, states for S must be an integer greater than one because a ''whorl'' does not refer to a pattern of complete strip reduction. For instance, in *D. proteus* the same number of strips, 18, line the anterior rim of the canal, surround the periphery of the cell, and meet at the posterior tip (Angeler, Müllner, and Schagerl 1999); therefore, $W_A = W_P = 0$. Consequently, there are no states for S. This state will hypothetically be found in plesiomorphically colorless euglenids

Table **2**. Symbols used to denote characters associated with the patterns of strips on euglenid pellicles.

Symbol	Character
C	Minimum number of strips surrounding the canal
P	Maximum number of strips around the periphery
T	Number of strips that meet at the posterior tip
W_A[a]	Number of whorls of strip reduction near the anterior end[c]
W_P[b]	Number of whorls of strip reduction near the posterior end[d]
S	Number of strips between consecutively terminating strips on a whorl
X	Number of strips immediately preceding a whorl of strip reduction

[a] Subscript ''A'' refers to ''anterior''.
[b] Subscript ''P'' refers to ''posterior''.
[c] A lowercase Roman numeral denotes each anterior whorl.
[d] An uppercase Roman numeral denotes each posterior whorl.

regardless of whether strips are arranged longitudinally or helically. In other taxa, the strip number is halved across a whorl and S = 1. Furthermore, the state for S may be different on consecutive whorls of a single cell. For instance, in *E. mutabilis*, S equals 3 on whorl I, 2 on whorl I′, and 1 on whorl II (Fig. 12).

We have recognized three primary patterns of strip reduction associated with the euglenid pellicle: exponential, pseudoexponential, and linear. The relative difficulty in scoring C, P, T, S, and W depends on the particular characteristics of each taxon. It is valuable to describe these relationships mathematically so that a character state that is difficult to score can be derived by the scores of the other characters. Mathematical definitions of organic patterns also have the quality of precision that allows us to recognize commonality and congruity between otherwise disparate natural phenomena (Thompson 1943). The general equations themselves may provide important insights for inferences about euglenid phylogeny.

It is clear that when S = 1, strips are reduced exponentially. For instance, following one whorl at either pole the strip number drops from P to P/2. Any exponential pattern of decay can be described by the following equation:

$$A = A_0\, e^{kt} \qquad \text{(Eq. 1)}$$

where A = the final state, A_0 = the initial state, t = any particular time during decay, and k = a rate constant. The rate constant ''k'' can be determined for any pattern of decay when both A_0 and A are known at a specific time ''t''. In regard to the reduction of pellicle strips at either pole, A_0 is equivalent to P; A is equivalent to either X, C, or T; t is equivalent to W; and k reflects the state for S, where S = 1. When t = 1, we know that A = P/2; and because we know that A_0 = P, we can solve for k. The value of k equals ln (P/2/P) · t, which is equivalent to ln (0.5). Replacement of the symbols in Eq. 1 with the characters dealing with patterns of strips leads to two separate equations: $T = P\, e^{kW_A}$ and $C = P\, e^{kW_P}$. Solving for W, which is often the most difficult character to score, leads to the following two equations that describe exponential patterns of strip reduction at both the anterior ($_A$) and posterior ($_P$) ends of the cell (Fig. 19).

$$W_A = 1/k \cdot \ln (C/P) \qquad \text{(Eq. 2)}$$

$$W_P = 1/k \cdot \ln (T/P) \qquad \text{(Eq. 3)}$$

Once the exponential patterns described by Eq. 2 and 3 were understood, we hypothesized that there were developmental constraints imposed on C, P, T and W. Hypothetically, W was dependent upon the number of times P was wholly divisible by two. For instance, a pellicle with P = 60 could have no more than W = 2 at either end of the cell (X_I = 60, X_{II} = 30, and T = 15). This hypothesis was surprisingly falsified after examining the posterior pellicle of *Euglena* sp. and some individuals of *E. gracilis.* For example, in some cells of *Euglena* sp. P = 56 and T = 4. Using data in Eq. 3, W = 3.8. However, direct examination of the pattern of strip reduction at the posterior end of *Euglena* sp. shows four discrete whorls; the exponential pattern of strip reduction breaks down on whorl IV (Fig. 10, Table 1). Therefore, pseudoexponential patterns of strip reduction can be identified when W is equivalent to some fraction after C, P, and T have been entered into either Eq. 2 or 3. The correct value for W, however, is the next integer rounded up from the fraction produced by the equation.

Even though the strips of most euglenids examined so far can be described by Eq. 2 and 3, we have also observed a linear pattern of strip reduction at the posterior end of *E. mutabilis.* In this taxon, S_P changes as the strips continue towards the posterior tip, which ensures that on each of three whorls, a constant number of strips terminates. This constant number of terminating strips is equivalent to the number of strips that meet at the posterior tip, namely T. Therefore, T is equivalent to the slope of a line. This pattern of strip reduction can be expressed using the standard equation for a line on a Cartesian coordinate system:

$$y = mx + b \qquad \text{(Eq. 4)}$$

Replacement of the symbols in Eq. 4 with those for strip characters leads to $P = TW_P + T$. Solving for W_P leads to $W_P = (P - T)/T$, which is equivalent to the following equation (Fig. 19):

$$W_P = P/T - 1 \qquad \text{(Eq. 5)}$$

Potentially, the slope may not equal T in other taxa with linearly reducing strips near the posterior end. In these cases, the slope may be described as $X_I - X_{I'}$. Therefore, the more general equation is:

$$W_P = (P - T)/(X_I - X_{I'}) \qquad \text{(Eq. 6)}$$

The exponential and linear patterns of strip reduction near

Table **3**. Patterns of pellicle pores in the euglenid pellicle. ''n'' refers to the frequency of the mode for the ''number of strips between pores'' over the number of cells observed. In the few cases when one pattern dominated another pattern on an individual cell, the dominant pattern was scored. The range of variation is shown parenthetically to the mode. When no consistent pattern was observed, modes were not reported.

	Taxon				
Character	*D. proteus* (n = 30/30)	*E. mutabilis* (n = 30/30)	*E. myxocylindracea* (n = 25/30)	*E. terricola* (n = 28/30)	*E. cantabrica* (n = 30/30)
Pores Present	no	yes	yes	yes	yes
Pore Density	—	sparse	sparse	abundant	abundant
Number of Strips between Pores	—	(2,4,8)	8(4,8)	4(2,4)	2

the posterior end are almost certainly evolutionarily derived from one another (not products of convergent evolution). It is hypothesized that the linear pattern of strip reduction is derived from an ancestral exponential pattern with two whorls (Fig. 20). A comparison of two familiar examples is worthwhile in order to both illustrate how one pattern can be derived from the other and justify the notation used to label the whorls in a linear pattern. *Euglena myxocylindracea* and *E. mutabilis* both posses 40 strips around the periphery (P = 40) and 10 strips that meet at the posterior tip (T = 10). However, in *E. myxocylindracea,* W_P equals two and S_P equals one within an exponential pattern, and in *E. mutabilis* W_P equals three and S_P equals three, two, and one, respectively, within a linear pattern (Fig. 12). If we assume that the first derived state was the exponential pattern of strip reduction (S = 1), then the linear pattern can be derived straightforwardly. Let whorl I of an exponential pattern with two whorls (like *E. myxocylindracea*) segregate into two separate yet homologous whorls. This can be accomplished by allowing every other terminating strip of whorl I to slide toward the anterior end relative to the initial position of the whorl. Consequently, a third and second whorl would be formed where S equals three and two, respectively (like *E. mutabilis*). However, because these two new whorls are derived from (homologous to) the segregation of whorl I within an exponential pattern of strip reduction, they are labeled whorl I and I′ within the linear pattern.

It is possible that the reverse scenario may have occurred. In this case, whorl I of a linear pattern of three whorls slid posteriorly until it overlapped with whorl I′, which caused S to equal one. However, this scenario is arguably less parsimonious because of insights gained from an identified character state series associated with the number of whorls present within an exponential pattern of strip reduction on different taxa (Fig. 20). It is hypothesized that zero whorls of strip reduction ($W_P = 0$) is the ancestral state. This is consistent with our observation that $W_P = 0$ for *D. proteus,* which diverges early within phylogenies based on SSU rDNA sequences (Preisfeld et al. 2000). Parsimoniously, an exponential pattern of strip reduction including one whorl ($W_P = 1$) evolved before an exponential pattern including two whorls ($W_P = 2$); likewise, an exponential pattern including two whorls ($W_P = 2$) evolved before an exponential pattern with three whorls ($W_P = 3$), and so forth (Fig. 20). A jump from one whorl in an exponential pattern to three whorls in a linear pattern is required in order for a linear pattern of strip reduction to have evolved prior to an exponential pattern with two whorls ($W_P = 2$).

The morphology of pellicle pores may also be significant phylogenetically. For instance, some euglenids have muciferous bodies with pellicle pores that are not visible under the SEM (e.g. *E. helicoideus, E. triqueter, L. buetschlii*; BSL, pers. observ.). Other taxa (*Colacium calvum*) have pores that are manifested as very subtle slits (Willey 1984). By contrast, the three taxa *E. cantabrica, E. terricola,* and *E. myxocylindracea* possess very conspicuous pellicle pores (Fig. 13, 15, 17). These data combined with discrete patterns of conspicuous pores may provide phylogenetic information for the recognition of clades.

The different patterns of pores described in this paper, namely rows of pores separated by two, four, and eight strips, demonstrate a character series where each state differs by a power of two (2^1, 2^2, and 2^3). Perhaps the functional unit of these helical pellicles is a pair of strips. We are aware of only a few euglenids with helical pellicles that possess a number of peripheral strips that is not wholly divisible by two (P = C = 15 in *Cryptoglena pigra*; Owens, Farmer, and Triemer 1988). Also interesting is that pairs of strips are involved with cell division. Just prior to cytokinesis, a complement of newly-formed strips (immature strips) emerges between adjacent existing strips (mature strips); that is, the number of strips is doubled during mitosis (e.g. Mignot, Brugerolle, and Bricheux 1987; Sommer and Blum 1965). The pellicle divides semiconservatively where strips rupture in pairs near both sides of the fork of a longitudinal cleavage furrow (Bouck and Ngo 1996; Mignot, Brugerolle, and Bricheux 1987). Two pairs of ruptured strips dangle on each daughter cell before the ruptured pairs fuse (zipper) and form two contiguous strips.

The rows of pores described in this paper may provide morphological markers for tracing the maturation of strips during cell reproduction. Moestrup and Hori (1989), for instance, have demonstrated how three cell divisions are necessary before a newly formed flagellum in an octoflagellate (*Pyramimonas*) can achieve the final mature state. Perhaps this sort of maturation process also occurs within the cytoskeleton of euglenids. It may be that strips just anterior to rows of pellicle pores (or vice versa) assume the final mature state and all other strips will achieve that state within subsequent daughter cells following a specific number of cell divisions. This intriguing possibility seems worthy of further experimentation.

Comparison of morphological data to molecular phylogenies. This paper lays down some of the groundwork for a contribution consisting of a molecular phylogeny based on SSU rRNA sequences and the phylogenetic mapping of pellicular character states. At present, only a few molecular phylogenies of euglenids are available for comparison (Linton et al. 1999; Linton et al. in press; Montegut-Felkner and Triemer 1997; Preisfeld et al. 2000; Thompson et al. 1995). With regard to the taxa that we have examined, only *D. proteus, E. gracilis* and *E. myxocylindracea* have had genes sequenced; the SSU rRNA and the *rbcL* genes have been sequenced for *E. gracilis,* the SSU rRNA gene has been sequenced for *D. proteus,* and the *rbcL* gene has been sequenced for *E. myxocylindracea.* Although the published gene trees do support that the ancestral state is $W_P = 0$, there is not yet enough taxonomic overlap to independently test our hypotheses about the evolution of whorls of strip reduction (Fig. 20) with molecular data.

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UPCOMING MEETINGS

The 14th Seminar on Amebiasis

November 27–30, 2000

El Colegio Nacional, Mexico City

Free communications are invited on all aspects of
Basic and Clinical Research on
Entamoeba histolytica and related *Entamoeba*

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See The Entamoeba Homepage web site:
http://www/lshtm.ac.uk/mp/bcu/enta/seminar.htm

WorldLeish II

International Congress on *Leishmania* and Leishmaniasis

May 20–24, 2001

Hersonissos, Crete, Greece

Inviting suggestions for the scientific program

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