A model for the morphogenesis of strip reduction patterns in phototrophic euglenids: evidence for heterochrony in pellicle evolution

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SUMMARY We propose a general developmental model that explains the evolutionary origin, diversification, and inheritance of pellicle strip patterns in phototrophic euglenids. Dividing cells of Euglena gracilis, E. viridis, and Phacus similis were observed with scanning electron microscopy in order to study the morphogenesis of posterior whorls of strip reduction. We found evidence that constant whorl numbers are maintained through cell division because of organized strip growth before and during cytokinesis. Alternating nascent strips form a new whorl of strip reduction at each of the anterior and posterior ends of daughter cells. Strips that terminated to form posterior whorls in the mother cell change in length during the development of daughter cells. In the mother cells of E. gracilis, the strips forming whorls I and II grow to become whorls II and III, respectively, in the daughter cells; the strips forming whorl III in the mother cell lengthen and meet with other strips already present at the posterior tip of daughter cells. This process of whorl morphogenesis during asexual reproduction is consistent with known variation in pellicle strip patterns and suggests that heterochrony played a major role in the ultrastructural evolution of phototrophic euglenids.

INTRODUCTION

Euglenids are predominantly free-living biflagellate eukaryotes that play important roles as consumers or producers in both marine and freshwater habitats; some feed on bacteria or other eukaryotes (phagotrophs), some absorb nutrients from the surrounding environment (osmotrophs), and others are capable of photosynthesis (phototrophs). Euglenids are closely related to kinetoplastids, together forming the two major subgroups within a larger clade of eukaryotes called the Euglenozoa. Kinetoplastids contain several lineages of small phagotrophs and important vertebrate parasites such as Trypanosoma spp., which can cause sleeping sickness and Chagas’ disease. Photosynthesis within the Euglenozoa is limited to one subclade of euglenids that resulted from a secondary endosymbiotic event wherein a green alga was engulfed by an ancestral euglenid and its plastid retained by the predator (Gibbs 1978). Subsequently, the phototrophs underwent extensive diversification and now exhibit a wide variety of ultrastructural characters that appear to be the direct or indirect result of plastid acquisition (Leander 2004). In particular, phototrophs have acquired a light sensing complex comprised of a carotenoid-rich eyespot and a paragflagellar body, have undergone extensive reduction of the complex feeding apparatus that is present in phagotrophic relatives and have been subject to major changes in the structure of their cytoskeleton, or pellicle.

The pellicle is composed of the plasma membrane, protein strips, subtending microtubules, and endoplasmic reticulum. The laterally articulating pellicle strips run longitudinally or helically from the anterior canal region to the posterior end of the cell and create a striated pattern on the cell surface (Fig. 1). The proteinaceous strips are of primary interest as their structure, number, and orientation have profound effects on the size, locomotion, and feeding habits of euglenids: for example, species that feed on other eukaryotes tend to have more numerous, helically arranged strips. This condition is thought to facilitate sliding between strips (Suzaki and Williamson 1985) and associated rapid changes in cell shape (metaboly) that is advantageous for engulfing large prey (Leander 2004). More derived lineages of phototrophs, on the other hand, have fewer, longitudinally arranged strips with complex lateral projections underneath the cell surface (Leander 2004). These features are conserved within species and varied enough to provide useful characters for taxonomic identification and phylogenetic inference (e.g., Leander et al. 2001; Brosnan et al. 2005).

In some euglenid species, pellicle strips terminate before reaching the posterior end of the cell. In phototrophic euglenids and osmotrophic taxa whose ancestors were phototrophs (“secondary osmotrophs” have lost photosynthesis), these terminations may form “whorled” patterns of reduction, so-called because the terminating strips form a circular
Before cell division, euglenids replicate the pellicle by doubling the number of strips (a value defined as “P”); Leander and Farmer 2000a; Fig. 1, A and B). Nascent strips form within the articulation zones between adjacent mother strips near the canal opening (the anterior end of the cell; Sommer and Blum 1965; Hofmann and Bouck 1976; Gillott and Triemer 1978; Dubreuil and Bouck 1985; Mignot et al. 1987; Bouck and Ngo 1996; Vismara et al. 2000). These nascent strips grow towards the posterior end as cell division progresses, and cytokinesis begins before nascent strips are fully grown. Two adjacent strips on each side of the canal are separated from one another and each is rejoined to one of the strips on the opposite side, forming a longitudinal cleavage furrow (Bouck and Ngo 1996; Fig. 1, C and D). In this way, the number of strips can be evenly divided between the two developing daughter cells. When symmetrical cell division is complete, each daughter cell possesses the same number of strips as the mother cell. Minor variations in the number of strips in different individuals within a taxon indicate that asymmetrical division also occurs, whereby strips are unevenly distributed between daughter cells (Leander and Farmer 2000a, 2001; Leander et al. 2001). However, the processes by which posterior strip reduction develops and is inherited from generation to generation are unclear and have never been articulated (Leander et al. 2001).

In order to understand the origin, development and inheritance of strip reduction, we observed dividing cells belonging to several phototrophic euglenids using scanning electron microscopy (SEM), namely *Euglena gracilis*, *E. viridis*, *Phacus similis*, and *P. segretti*. Our work concentrated on dividing cells of *E. gracilis*, a taxon with three exponential whorls of posterior strip reduction. We were able to determine the fate of the exponential whorl formed by nascent strips, the sequence of whorl morphogenesis and the fates of the whorls already present in the mother cell. We synthesized these data and propose a model of whorl morphogenesis that explains not only the inheritance of consistent strip reduction patterns, but also provides evidence that heterochrony has played a major role in the evolution of the euglenid cytoskeleton.

**MATERIALS AND METHODS**

The following cultures were used in this investigation: *E. gracilis*, *E. viridis* (SAG 1224-17d), *P. segretti* (ACOI 1337), and *P. similis* (SAG 58.81). *E. viridis* and *P. similis* were purchased from Sammlung von Algenkulturen Göttingen (SAG); *P. segretti* was purchased from the Coimbra Collection of Algae (ACOI); *E. gracilis* was obtained from the Biology Program at the University of British Columbia. *E. viridis* and *P. similis* were grown in modified MES-volvox medium (Provasoli and Pinter 1959); *P. segretti* was grown in LM7 medium (http://www.uc.pt/botanica/
ACOI_M~1.htm). *E. gracilis* cells were grown in a standard *Chlamydomonas* medium (recipe available upon request) and exposed to a 12 h light/12 h dark cycle at 18°C and 17°C, respectively. *E. gracilis* cells were harvested for SEM 4.5 h after commencement of the dark cycle. All cells were fixed with osmium tetroxide according to Leander and Farmer (2000a), placed on Millipore filters and dehydrated with a series of increasing ethanol concentrations. Cells were critical point dried with CO2, mounted on aluminum stubs and sputter coated with a mixture of gold and palladium. Samples were viewed using a Hitachi S4700 scanning electron microscope. One dividing cell each of *P. similis*, *P. segretti*, and *E. viridis*, and 42 cells of *E. gracilis* in various stages of cell division were observed in order to help substantiate our model.

**RESULTS AND DISCUSSION**

**Whorl I is formed by nascent strips**

The developing, nascent strips in dividing *P. similis* alternate between mature strips (Fig. 2A), forming a whorl of exponential reduction anterior to the terminating strips present in the mother cell. During interphase in *P. segretti* (*Wp* = 2), strips that terminate at the anterior canal, forming an anterior whorl of reduction, also terminate before reaching the posterior cell tip and form the first posterior whorl of strip reduction (Fig. 2B). These strips are inferred to have been formed during the last round of cell division, which is consistent with reports that nascent strips form alternating “minor” strips within the canal in the secondary osmotroph *Cyclidiopsis acus* (Mignot et al. 1987). In other words, the strips that terminate before entering the canal in phototrophic euglenids (and secondary osmotrophs) are inferred to be the developmental equivalents to the minor strips identified by Mignot et al. (1987) in *C. acus*, and thus are more recently formed than the neighboring strips that do enter the canal. During late cytokinesis in *E. viridis*, the anterior-most exponential whorl of reduction is visible near the point where the cells are joined at their posterior tips (Fig. 2C). The strips forming this whorl of reduction are narrower than several of their neighbors, indicating that they are younger and have been produced immediately before cell division. The widest strips, on the other hand, all extend to where the cells are joined at their posterior tips.

**Fig. 2.** Evidence that whorl I in phototrophic euglenids is formed by nascent pellicle strips. (A) A *Phacus similis* cell beginning to divide. The nascent strips (arrowheads) are still growing towards the posterior end of the cell and appear to form an exponential whorl of reduction (asterisks); the cleavage furrow (arrow) has already begun to form. Bar = 5 μm. (B) *P. segretti* at interphase. Alternating strips (arrowheads) that terminate at the anterior canal (on the left) also terminate to form the first whorl of posterior reduction (inset, asterisks; bar = 5 μm). (C) The cleavage furrow between two developing *Euglena viridis* cells near the end of cytokinesis. The two daughter cells are joined only at the posterior tip. Alternating strips (arrowheads), inferred from their relatively narrow width to be nascent, form an exponential whorl of reduction on each daughter cell (asterisks) (bar = 2 μm).
Fig. 3. Progression of the cleavage furrow and position of furrow strips during cell division in *Euglena gracilis*. Double arrows indicate separating furrow strips while arrows indicate rearticulating furrow strips; arrowheads indicate nascent strips and their direction of growth. Bars = 1 μm. (A) Anterior view showing the beginning of cytokinesis. The two pairs of furrow strips, m (mature) and n (nascent) on the left, and m' and n' on the right, have separated and rearticulated with strips from the opposite side of the cell: m' with n, and m with n'. Each daughter cell has 32 strips surrounding the canal. (B) Strips m and m' are further separated from n and n', respectively, and rearticulated with n' and n, respectively. The anterior ends of two daughter cells are now distinctly formed: the cell on the left has 40 strips; the cell on the right has 36 strips. (C) As cytokinesis progresses, the cleavage furrow appears to align with or surpass the extent of growth of the nascent furrow strips, n (located behind the cell) and n'. (D) A diagram summarizing the changing positions of furrow strips during cytokinesis. Furrow strips have been coded as follows: m' is dark gray, m is mid-gray, n' is light gray, and n is white-gray. After strip doubling, m is adjacent to n and m' is adjacent to n'. The pairs of furrow strips are located opposite to one another, with the anterior canal between them. Cleavage furrow formation begins at the anterior of the cell, where m separates from n and m' separates from n'. Where the strips have separated, m rearticulates with n' and m' rearticulates with n so that the anterior canal is divided into two. Separation of m and n, and m' and n' continues towards the cell's posterior end, while rearticulation between m and n' and m' and n continues in a corresponding fashion. As these processes take place, the cleavage furrow progresses and the two daughter canals are further separated from one another. Upon completion of cytokinesis, m' is located adjacent to n on one daughter cell, and m is located adjacent to n' on the other daughter cell.
furrow strips from the opposite side of the cell. As in the separation process, the rearticulation process necessarily progressed from the anterior end of the cell toward the posterior end. In specific terms, the nascent furrow strips, n and n', articulated with the mature strips on the opposite side of the cell such that n articulated with m' and n' articulated with m (Fig. 3).

As the opposite pairs of strips separated and then rearticulated (Fig. 3), a cleavage furrow progressed from the anterior end to the posterior end of the cell, gradually forming two daughter cells. The rate of cleavage furrow formation actually exceeded that of nascent strip growth (Fig. 3C), which required the separation of two adjacent mature strips along their lateral articulation zone near the posterior end of the cell. Upon the completion of cytokinesis, however, one daughter cell inherited strips n and m' whereas the other daughter cell inherited strips n' and m (Fig. 3D). This process of inheritance maintained the alternating pattern of nascent and mature strips.

In their study of cell division in the secondary osmotroph _C. acus_, Mignot et al. (1987) observed that nascent strips arose from a “morphogenetic center” in the articulation zone between the “overhang” of one strip and the “hook” of another (for definitions of ultrastructural terms, see Leander and Farmer 2001a). In this context, we can deduce that the nascent strips, such as n and n', develop beneath the overhangs of adjacent mature strips, such as m and m', respectively. It is also known that some of the microtubules underlying the nascent furrow strips are inherited from their respective mature furrow strips (Mignot et al. 1987). This developmental linkage and relative positioning of nascent furrow strips to mature furrow strips ensures that rearticulation occurs between a nascent and a mature strip in each daughter cell, which maintains consistent patterns of posterior strip reduction from one generation to the next.

Moreover, this consistency relies on the cleavage furrow developing between a mature strip and the nascent strip that developed from it; it is helpful to point out that the nascent strips are always on the right hand side of the mature strips from which they developed when viewed from the anterior end. The following hypothetical exercise reinforces the importance of the position of the mature furrow strips relative to that of the nascent ones in transferring a consistent pattern of strips from mother cell to daughter cells. If for example (i) the position of n' was reversed and located to the left of m' when viewed from the anterior end, (ii) n remained to the right of m and (iii) n' and m' were separated along their lateral articulation zones, then rearticulation would occur between n and n' and m and m'. This would cause adjacent nascent strips n and n' to terminate in whorl I in one daughter cell and adjacent mature strips m and m' to continue past whorl I in the other daughter cell. This outcome would be inconsistent with the exponential and linear patterns of strip reduction observed in phototrophic euglenids so far.

The relative ages of the furrow strips may be important in determining the initial location of strip separation and the formation of the cleavage furrow. Unfortunately, with the SEM methods described here, the relative ages of m and m' are indeterminable without observing the posterior whorls of the cell and the fates of furrow strips simultaneously. In cells where the posterior whorls were visible, the cleavage furrow was not, which prevented us from identifying the furrow strips. Because strip age plays an important role in pellicle strip development, as evidenced by differences between canal strips (Mignot et al. 1987) and the morphogenesis of posterior patterns of reduction described here, determining the relative age of furrow strips might provide important further insight into pellicle morphogenetic processes and the evolutionary history of pellicle characters. An antibody labeling experiment using different sized latex beads, as an extension of the experiments conducted by Hofmann and Bouck (1976), wherein cells are allowed to duplicate their strips and divide after the labeling procedure, could prove useful for this purpose.

Interestingly, the location of the cleavage furrow did not always permit even distribution of strips between daughter cells. Two cells (Fig. 3, A and B) in the early stages of cytokinesis had fewer strips than would be expected from cells whose interphase _P_ value is 40. One (Fig. 3A) had _P_ = 64 strips and the other (Fig. 3B) had _P_ = 76 strips, indicating that the mother cells during interphase had _P_ = 32 and _P_ = 38, respectively. Moreover, strips were divided evenly between the daughter cells in the first instance, yielding two daughter cells with 32 strips each (Fig. 3A), whereas strips were unevenly divided in the second instance, yielding one daughter cell with 36 strips and one with 40 strips (Fig. 3B). Whether or not this is due to an inherent “deterministic” property present in the mature furrow strips m and m' that is lacking in other pellicle strips, remains to be investigated.

**Posterior strip reduction patterns in _E. gracilis_**

In _E. gracilis_, the mode for _P_ (the number of strips around the cell periphery) was 40 (range = 32–40, _n_ = 17). All observed cells had an even number of strips. Cells exhibited an exponential pattern of posterior strip reduction with three whorls (Fig. 4A). When exponential reduction was prevented by insufficient strip number (in this case, a number that cannot be exponentially reduced three times, such as 36 or 38), pseudo-exponential reduction, where strips terminate asymmetrically in the most posterior whorl, was observed (Leander and Farmer 2000a).

Nascent strips formed at the anterior end of the cell and grew towards the posterior tip (Fig. 4B). These new strips were narrower than mature strips (Fig. 4, C and D) and alternated between them. Cells commenced cytokinesis before
nascent strips reached the posterior tip or the position of the first whorl of reduction (Fig. 4, C–F). Between the beginning and end of cytokinesis, cells had four (Fig. 4D) whorls of exponential reduction. At the end of cytokinesis, cells were joined just at their posterior tips and had three whorls of exponential reduction (Fig. 4F).

Multigenerational strips and posterior whorls of reduction: a model of inheritance

Although nascent strips have been known to be evenly distributed between daughter cells for some time (Hofmann and Bouck 1976), it was not clear how different generations of...
strips are incorporated into the defined patterns of posterior reduction that are conserved within phototrophic euglenid taxa. Here we propose a model of whorl morphogenesis that incorporates semiconservative pellicle strip inheritance and explains how a pattern of posterior strip reduction can be maintained through successive cell divisions in a taxon or cell lineage. It requires, however, that mature pellicle strips be capable of repeated elongation events after their initial synthesis. Mignot et al. (1987) found that this is exactly what occurs to half of the mature strips within the anterior canal of the secondary osmotroph *C. acus*. Before division in *C. acus*, the 16 minor strips that alternate between 16 major strips grow to the same size as the major strips. Subsequently, 32 nascent strips emerge between the 32 mature strips and grow to become the (16) minor strips in each of the daughter cells.

Likewise, our model proposes that the morphology (namely, the length) of strips at the posterior end of the cell also changes from generation to generation. In the example outlined in Fig. 5, a cell with $P = 32$ and $W_p = 1$ (i.e., one whorl of exponential reduction composed of 16 terminating strips) doubles its strips before cell division so that $P = 64$. Because nascent strips alternate with mature strips, the cell immediately before cytokinesis has two exponential whorls, $W_p = 2$. The cell begins to divide before the nascent pellicle strips are fully grown. As nascent strips grow longer and the cleavage furrow progresses further toward the posterior of the cell, the strips forming whorl I in the mother cell grow longer as well. This causes whorl I to move toward the posterior tip of the cell and gradually disappear as the strips forming whorl I achieve the same length as the strips that reached the

![Diagram of whorl morphogenesis](image)

**Fig. 5.** A model for the maintenance of whorls of reduction on the posterior end of dividing euglenids. A hypothetical cell with a total strip number of 32 has one exponential whorl of reduction (white circle) composed of 16 pellicle strips (dark green) and 16 strips (light green) reaching the tip of the cell. During strip doubling prior to cell division, 32 nascent strips (yellow) extend from the canal towards the posterior end of the cell and form a second whorl of reduction (red circle). As the nascent strips continue to grow, the strips composing the original whorl of reduction begin to grow towards the posterior tip. The cell body begins to divide before the nascent strips are finished growing. After cell division pellicle strips are distributed evenly between two daughter cells: each cell has 16 mature strips (green) that were present in the mother cell, extending to the posterior tip, and 16 nascent, terminating strips (yellow) that form the new whorl of reduction. Eight of the strips at the posterior tip (dark green) belonged to the posterior whorl of reduction in the last generation. $P$, total strip number; $W_p$, number of posterior whorls of reduction.
posterior tip in the mother cell. The nascent strips grow to reach the former position of whorl I, so that when division is complete each daughter cell has $W_p = 1$ like the mother cell. However, whorl I is now composed of a new generation of nascent strips, and the strips that formed whorl I in the previous generation now reach the posterior tips of the daughter cells and are intermixed with the strips formed in earlier generations.

This model is applicable to taxa with more than one posterior whorl, such as *E. gracilis*. During cell division, strips forming whorl III grow to the same length as the strips that reach the posterior tip; strips forming whorl II grow to the same length as the strips that formed whorl III; strips forming whorl I grow to the same length as the strips that formed whorl II; and nascent strips grow to the same width and length as the strips that formed whorl I (Fig. 6). Because each of these groups of strips contains twice as many strips as the set immediately posterior to it, this growth process results in a cell with twice as many strips and three exponential whorls of reduction, each containing twice as many terminating strips as the whorls in an interphase cell (although cell division begins before the growth process is complete). When cell division is complete, each daughter cell possesses the same number of strips and the same pattern of reduction as the mother cell. Thus, each whorl of reduction is composed of terminating strips belonging to different generations produced during previous cell divisions: the strips in whorl I were produced during the most recent round of cell division, the strips in whorl II are one generation older, and the strips in whorl III belong to a still older generation. The strips that reach the posterior tip of the cell are composed of at least two different generations (because during the last round of cell division younger strips from whorl III grew between and intermixed with the older strips already present at the tip). Strictly speaking, there can be as many as three or four generations represented by strips at the tip because whorl III strips are incorporated between tip strips at each cell division. For the sake of simplicity, however, tip strips in interphase cells are

**Fig. 6.** A model of posterior whorl morphogenesis in *Euglena gracilis*. A mother cell has three posterior whorls of exponential reduction: whorl I (red), whorl II (green) and whorl III (blue). Before cell division, alternating nascent strips develop; as they lengthen towards the posterior end of the cell they form a fourth exponential whorl of reduction (yellow). Before the nascent strips are fully grown, the cleavage furrow and two daughter cells begin to form; the nascent whorl is now relatively close to the posterior of the cell. As the cleavage furrow progresses, the nascent whorl is disrupted and divided equally between the forming daughter cells. Whorl III is lost as the terminating strips forming it grow toward, and eventually reach, the posterior tip of the cell. The strips forming whorls I and II also grow slightly and are divided by the cleavage furrow, so that by the time cytokinesis is complete each daughter cell possesses three exponential whorls of reduction like the mother cell. In the daughter cells, whorl I is formed by nascent strips (yellow), whorl II is formed by strips that constituted whorl I in the mother cell (red), and whorl III is formed by strips from whorl II in the mother cell (green). Inset: *E. gracilis* with strips colored according to their relative age (uncolored strips are the oldest; bar = 2 μm).
color-coded as the same generation in all figures. Any inter-
phase *E. gracilis* cell can therefore have strips representing up
to six or seven generations: three generations represented by
posterior whorls of reduction, and three or four generations
represented by strips reaching the posterior tip of the cell (Fig.
6, inset).

**Heterochrony and the diversity of posterior strip
reduction patterns**

Posterior patterns of pellicle strip reduction are indicators
of phylogenetic relationships, and the ancestral state for
euglenids is the absence of strip reduction altogether (Leander
and Farmer 2000a; Leander et al. 2001; Fig. 7). According to
our model, the origin of a whorled pattern of strip reduction
involved the incomplete growth of nascent strips before di-
vision. That is, if the nascent strips failed to grow to the
posterior tip, then each daughter cell would have one exponen-
tial whorl of strip reduction, assuming that all of the nas-
cent strips terminated at the same point along the length of
the cell. If the differential growth of nascent strips was re-
peated during subsequent rounds of cell division, then mul-
tiple whorls of exponential strip reduction could be produced
(Fig. 7). This extension of the model is consistent with pre-
vious observations of different states for the number of pos-
terior whorls of strip reduction: one whorl (e.g., *E. cantabrica*,

![Diagram](image)

**Fig. 7.** Pathways for the hetero-
chronic evolution of posterior
patterns of strip reduction in pho-
totrophic euglenids. An ancestral
 cell without posterior whorls of re-
duction could give rise to a cell with
one exponential whorl of reduction
if nascent strips did not completely
extend to the posterior tip of the cell
before cytokinesis. Through several
repetitions of this event, cells with
two, three, and four exponential
whorls of reduction could be pro-
duced through time. Differences in
growth rate between alternating
strips within one exponential whorl
of reduction, depending on their
magnitude, could give rise to a
staggered exponential whorl or to
two linear whorls (which, though
apparently separate, would both be
formed by strips of the same gen-
eration). In this way an ancestral
cell with one exponential whorl of
reduction could give rise to a cell
with a pseudolinear pattern of re-
duction. Alternatively, the ancestral
state could give rise to pseudolinear
reduction directly, which could,
through homogenization of growth
rate of nascent strips, give rise to
one “clean” exponential whorl of
reduction. An ancestral cell with
two exponential whorls of reduc-
tion could give rise first to cells with
two posterior whorls of reduction,
one of which is staggered, then to
cells with three whorls of linear re-
duction. A cell with three exponen-
tial whorls of reduction could
likewise give rise to cells with two
staggered whorls of reduction,
which would in turn give rise to
cells with five bilinear whorls of re-
duction. These character state
changes are inferred to be rever-
sible, as indicated by backward ar-
rows.
Leander et al. 2001), two whorls (e.g. *E. laciniata*, Leander et al. 2001), three whorls (e.g. *E. longa*, Leander et al. 2001) and four whorls (e.g., *E. rustica*, Leander and Farmer 2000a; Brown et al. 2002). Moreover, differences in growth rate within one whorl of exponential reduction, such that every other terminating strip grew longer than its terminating neighbor, would result in a pseudolinear pattern of reduction, as observed in *Eutreptia pertyi* (Leander et al. 2001) (Fig. 7). Differential growth in whorl I of a cell with two exponential whorls of reduction would result in cells with a staggered exponential whorl, followed by a uniform exponential whorl. From this intermediate state, cells with three linear whorls of reduction, such as *E. mutabilis*, could be produced (Leander and Farmer 2000a). Likewise, an ancestral cell with three exponential whorls of reduction could give rise to cells with two staggered exponential whorls and one uniform exponential whorl (e.g., *Lepocinclis oxyuris*, Leander et al. 2001). Cells exhibiting this state in turn could yield descendants with five bilinear whorls (e.g. *E. helicoideus*, Leander and Farmer 2000b) (Fig. 7).

Some phylogenetic analyses of morphological characters and nuclear ribosomal RNA gene sequences (SSU and LSU rDNA) have suggested that a pseudolinear pattern of posterior strip reduction evolved before one “clean” exponential whorl. *E. pertyi*, for instance, is among the earliest diverging phototrophic taxa and has a pseudolinear pattern of posterior strip reduction (Leander et al. 2001) (Fig. 7). It should be emphasized that a pseudolinear pattern is effectively a disorganized version of one exponential whorl of strip reduction (Fig. 7). However, the origin of one “clean” exponential whorl of strip reduction by a single generation of aberrant nascent strips is also a plausible hypothesis for the ancestral state in phototrophic euglenids. Taxa known to have one exponential whorl of reduction include *E. cantabrica* (Leander et al. 2001), *Phacus oscillans* (Leander et al. 2001; Leander and Farmer 2001b), *Lepocinclis salina* (Conforti and Tell 1983) and members of the loricate genus *Trachelomonas* (Brosnan et al. 2005). The current molecular phylogenetic framework for euglenids, however, suggests that all of these taxa diverged relatively recently (Brosnan et al. 2003; Marin et al. 2003). Nonetheless, it cannot be ruled out that we have not yet observed early-branching taxa with a single exponential whorl of strip reduction because of low taxon sampling or the extinction of taxa possessing the ancestral character states.

The contentious phylogenetic position of *E. mutabilis* is problematic because its unique linear pattern of strip reduction has been used as evidence for its affinity to the Eutreptiales when molecular data were inconclusive (Leander et al. 2001). Although *E. mutabilis* can branch relatively early (i.e., subsequent to the Eutreptiales) in molecular and morphological phylogenies (Leander et al. 2001; Marin et al. 2003; Nudelman et al. 2003), it does not always do so (Marin et al. 2003; Nudelman et al. 2003). The SSU and LSU rDNA sequences from *E. mutabilis* are highly divergent, which has led to long-branch attraction artifacts in previous analyses (Leander et al. 2001; Brosnan et al. 2003; Marin et al. 2003). The evolutionary pathway of whorl reduction proposed by Leander et al. (2001) inferred from mapping this character on to a tree that may be affected by long-branch attraction and inadequate taxon sampling seems unsatisfactory in light of our developmental data. It should be noted, however, that according to our model the first two whorls in a species with linear reduction (i.e., whorls I and I’, produced by differential growth within whorl I) should be produced by one generation of nascent strips (Fig. 7). Furthermore, these whorls must coalesce during the next round of cell division to produce whorl II. If a cell had a pseudolinear pattern of posterior reduction, than the coalescence of whorls I and I’ (rather than their growth to the posterior end of the cell) to form whorl II and the production of whorls I and I’ by nascent strips during a single cell division event could result in daughter cells with three linear whorls of reduction. The multigenerational nature of the pellicle means that every other nascent strip is produced adjacent to a mature strip belonging to a different generation than its neighbors. Therefore, staggered and pseudolinear patterns of strip reduction are consistent with our model because it is possible that the relative ages of adjacent mature strips affect the growth rate of the nascent strips (Fig. 7). Morphological analyses of more phototrophic euglenid taxa, especially those belonging to the Eutreptiales, in the context of comprehensive molecular phylogenies (e.g., Marin et al. 2003) should help clarify these inferences.

**CONCLUSIONS**

Although the data presented in this paper provide a model for the maintenance of posterior whorls of strip reduction and compelling evidence for heterochrony in the evolution of this character, several key questions remain unanswered. The genetic basis for patterns of strip reduction is completely unknown. It remains to be discovered what triggered a change in morphogenetic processes and therefore posterior patterns of strip reduction. Knowledge of the genetic basis of whorl morphogenesis could help us to determine if the evolutionary pathways in Fig. 7 are reversible; this information could lead to a set of probabilities associated with the direction of character evolution that could be applied to phylogenetic analyses of morphological data using likelihood methods (Lewis 2001; Hibbett 2004). We are only beginning to understand developmental processes in single-celled eukaryotes in a framework that incorporates our increasing understanding of the vast phylogenetic and architectural diversity exhibited by these organisms. Research focusing on these processes could provide significant insights into the foundations of cellular differentiation and organismal diversity.
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