Macroevolution of complex cytoskeletal systems in euglenids

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Summary

Euglenids comprise a group of single-celled eukaryotes with diverse modes of nutrition, including phagotrophy and photosynthesis. The level of morphological diversity present in this group provides an excellent system for demonstrating evolutionary transformations in morphological characters. This diversity also provides compelling evidence for major events in eukaryote evolution, such as the punctuated effects of secondary endosymbiosis and mutations in underlying developmental mechanisms. In this essay, we synthesize evidence for the origin, adaptive significance and diversification of the euglenid cytoskeleton, especially pellicle ultrastructure, pellicle surface patterns, pellicle strip number and the feeding apparatus. We also highlight holes in our knowledge that must be filled before we are able to confidently describe euglenid cell biology and infer the earliest stages in euglenid evolution. Nonetheless, by possessing combinations of characters resulting from adaptive change and morphostasis, euglenids have retained key pieces of evidence necessary for reconstructing the early evolution and diversification of eukaryotic life. *BioEssays* **29:987–1000, 2007. © 2007 Wiley Periodicals, Inc.**

Introduction

Microbes are often thought of as lacking substantial morphological variation, but this is not true even for bacteria and certainly not for unicellular eukaryotes. The cells of eukaryotes are distinctive in possessing a nucleus, an endomembrane system, a complex cytoskeleton involved in feeding and locomotion and mitochondria (or hydrogenosomes), which are modern-day descendants of proteobacterial endosymbionts.[1] Several groups of eukaryotes also contain cyanobacterial endosymbionts called "plastids" (e.g. chloroplasts)

and are capable of photosynthesis. The origin of plastids in eukaryotes involved phagotrophic cells that engulfed and retained cyanobacterial prey, a process called "primary" endosymbiosis. The subsequent evolutionary history of photosynthesis in eukaryotes is exceedingly convoluted and involved at least three independent endosymbiotic events between phagotrophic eukaryotes and eukaryotic prey cells that already contained primary plastids (e.g. green algae and red algae).[2,3] Once photosynthesis was established in a previously phagotrophic cell, the evolutionary pressures on the cytoskeletal systems involved in locomotion and feeding changed. This gave rise to fundamental modifications of cell structures found in the descendants of these chimeric cells. Therefore, the cells of microbial eukaryotes consist of complex ultrastructural systems that reflect complex evolutionary histories, and the staggering diversity found in many groups of microbial eukaryotes makes them excellent systems for studying major innovations and structural transformations associated with the overall evolutionary history of organisms.

This essay focuses on a large group of eukaryotic microbes, namely the "Euglenida", that epitomizes the main points in the preceding discussion. The morphological diversity found in euglenids offers an extraordinary opportunity for assembling compelling inferences about cell character evolution (Fig. 1). For instance, comparative analyses of living euglenids have demonstrated some clear-cut examples of morphostasis and character states that comprise the intermediate components of structural transformation series.[4–6] These studies have also uncovered patterns of morphological diversity that shed considerable light on developmental mechanisms involved in the morphological diversification of the group.[7,8] However, perhaps most importantly, recent studies on euglenid biodiversity have identified many areas of uncertainty and deep holes in our overall knowledge that must be filled before we are able to confidently describe euglenid cell biology and infer the earliest stages in euglenid evolution. The main objective of this essay is to summarize our current understanding of euglenid character evolution, especially trends in the evolution of cytoskeletal systems. This narrative will also touch on several broader themes in macroevolutionary biology, such as (1) the punctuated effects of secondary endosymbiosis and underlying developmental mechanisms, (2) demonstrations of "reducible complexity" and (3) the

realization that fossils are a luxury in these endeavors, rather than a necessity.

Euglenid biodiversity and phylogeny

The widespread use of molecular tools and modern phylogenetic methods over the past few decades, has greatly improved our understanding of eukaryotic microbial diversity and interrelationships.[9] Reconstruction of the internal phylogenetic topology of euglenid diversity has been addressed most extensively using nucleotide sequences amplified from ribosomal genes (i.e. small and large subunit rRNA genes).[10–15] Although these genes have been helpful in resolving the phylogeny of some more recently diverged euglenids, they do not provide satisfactory phylogenetic signal
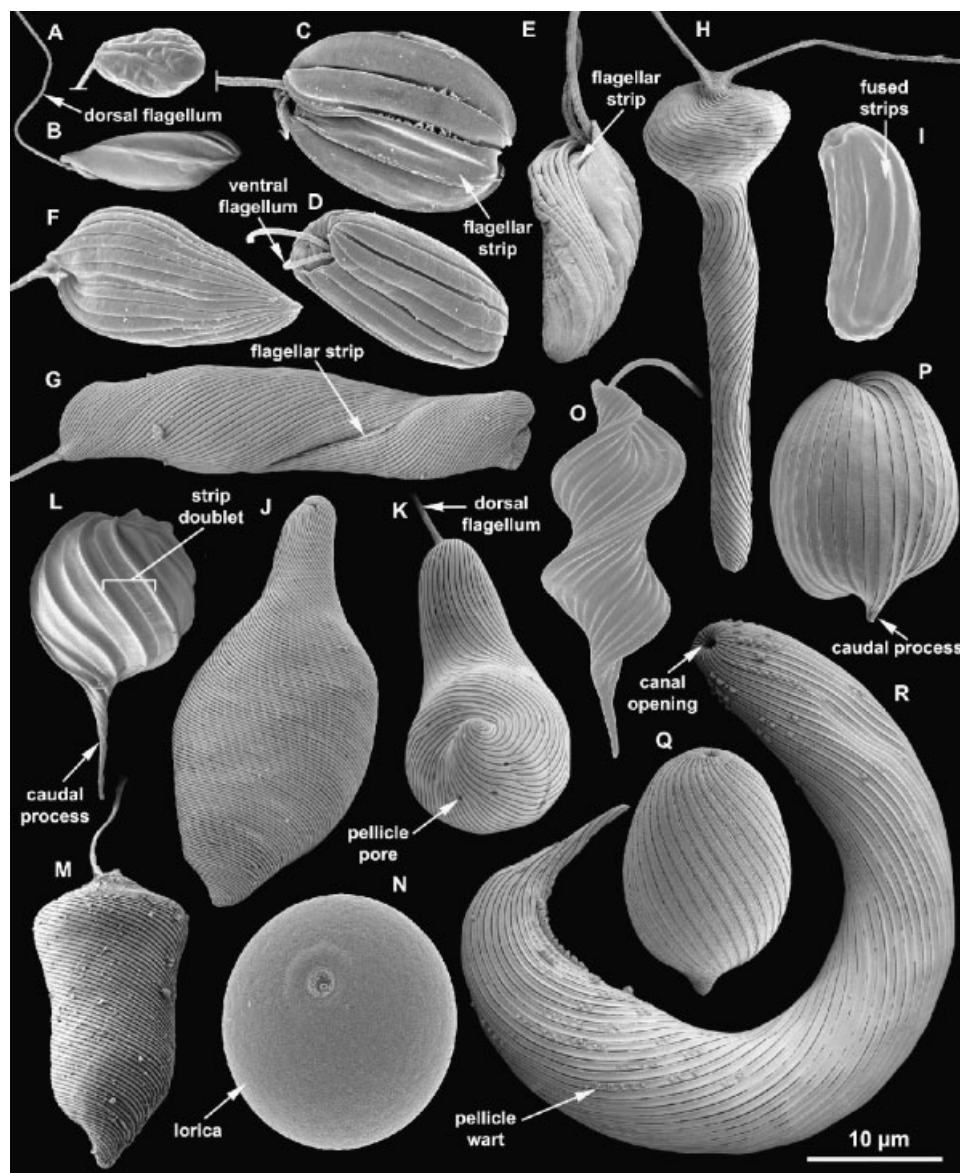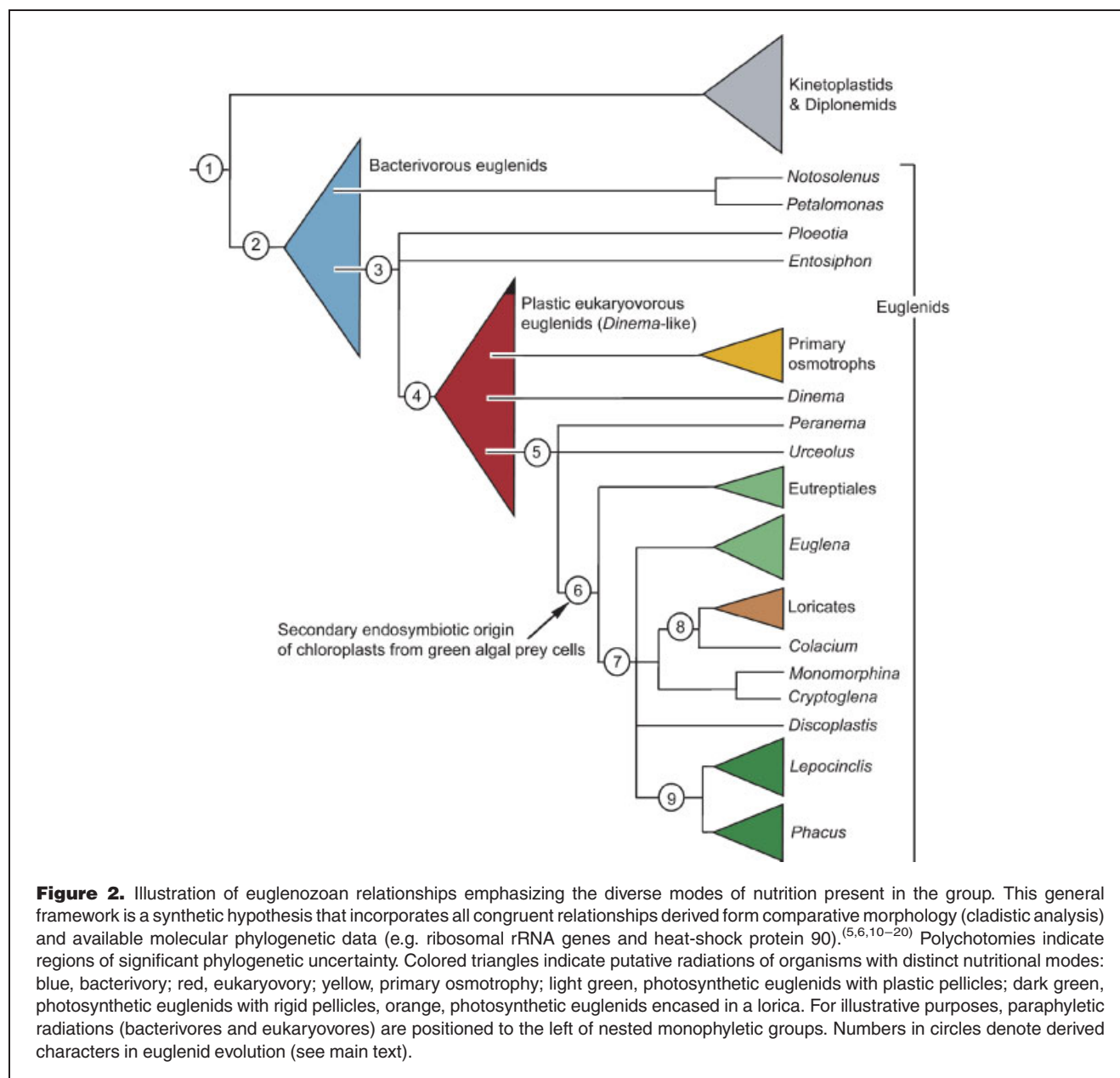

Figure 1. A synopsis of euglenid diversity using scanning electron microscopy (SEM). **A**: *Petalmonas* sp. with four pellicle strips, **B**: *Petalmonas cantuscygny* with eight pellicle strips, **C**: *Ploeotia* sp. with 10 pellicle strips, **D**: *Entosiphon sulcatum* with 12 pellicle strips, **E**: *Dinema sulcatum* with 20 pellicle strips, **F**: *Dinema* sp. with 20 pellicle strips, (**G**: *Peranema trichophorum* with 50 pellicle strips, **H**: *Distigma proteus* with 20 pellicle strips, **I**: *Rhabdomonas* sp. with eight fused pellicle strips, **J**: *Euglena mutabilis* with 40 pellicle strips, **K**: *Euglena* sp. with forty pellicle strips, **L**: *Monomorphina ovata* with sixteen pellicle strips, **M**: *Colacium mucronatum* with 40 pellicle strips, **N**: the lorica of *Trachelomonas* sp., **O**: *Phacus* sp. with 32 pellicle strips, **P**: *Phacus acuminata* with 32 pellicle strips, **Q**: *Lepocinclis ovum* with 32 pellicle strips, and **R**: *Lepocinclis spirogyra* with 36 pellicle strips. All images at the same scale.

at deeper levels in the phylogeny (Fig. 2). The most-compelling evidence for deep-level phylogenetic relationships of euglenids comes from comparative analyses of morphological data and some nucleus-encoded protein genes (e.g. heat-shock protein 90 and paraxonemal rod genes).[5,6,16−20] These data also indicate that euglenids fall within a putative eukaryotic supergroup called the Excavata, which includes a huge range of free-living and parasitic eukaryotic microbes, such as kinetoplastids (e.g. *Bodo* and *Trypanosoma*), diplomonads (e.g. *Giardia* and *Hexamita*), parabasalids (e.g. *Trichomonas* and *Trichonympha*) and oxymonads (e.g. *Saccinobaculus* and *Streblomastix*).[21,22]

Nonetheless, the Euglenida is a large group of single-celled flagellates with diverse modes of nutrition. Many of the approximately 1,000 described species of euglenids thrive within the interstitial spaces of marine and freshwater sediments and are considered "phagotrophic" because they hunt and ingest particulate food, such as bacteria and other microbial eukaryotes living in these environments. Phagotrophic euglenids employ a distinctive mode of gliding locomotion using two heterodynamic flagella: (1) a linear dorsal flagellum extends anteriorly and (2) a linear ventral flagellum extends posteriorly beneath, and often beyond, the cell and within a ventral groove (syn. "sulcus" or "flagellar strip") (Figs 1A−G,

Figure 2. Illustration of euglenozoan relationships emphasizing the diverse modes of nutrition present in the group. This general framework is a synthetic hypothesis that incorporates all congruent relationships derived form comparative morphology (cladistic analysis) and available molecular phylogenetic data (e.g. ribosomal rRNA genes and heat-shock protein 90).[5,6,10−20] Polychotomies indicate regions of significant phylogenetic uncertainty. Colored triangles indicate putative radiations of organisms with distinct nutritional modes: blue, bacterivory; red, eukaryovory; yellow, primary osmotrophy; light green, photosynthetic euglenids with plastic pellicles; dark green, photosynthetic euglenids with rigid pellicles, orange, photosynthetic euglenids encased in a lorica. For illustrative purposes, paraphyletic radiations (bacterivores and eukaryovores) are positioned to the left of nested monophyletic groups. Numbers in circles denote derived characters in euglenid evolution (see main text).

2—position 1). Each flagellum bears a single row of hairs (syn. mastigonemes) and is reinforced by a robust paraxial rod that runs in parallel to the microtubular axoneme (Fig. 2—position 1). The flagellar hairs and paraxial rods provide the underlying machinery required for gliding locomotion.[23] Phagotrophic euglenids that primarily consume bacteria are sometimes referred to as "bacterivores" (e.g. *Petalomonas, Ploeotia* and *Entosiphon*; Fig. 1A–D), and those that routinely ingest much larger prey items, such as other eukaryotic cells (e.g. diatoms and green algae), are sometimes referred to as "eukaryovores" (e.g. *Dinema, Peranema* and *Urceolus*; Figs 1E–G, 2—position 4).[4,6,24,25] Ultrastructural differences between bacterivores and eukaryovores, such as the organization of the feeding apparatus and the relative plasticity of the cell, reflect these different modes of nutrition. Morphological and molecular phylogenetic evidence indicate that rigid bacterivorous euglenids constitute the ancestral stem group from which plastic eukaryovorous euglenids evolved (Fig. 2).[4–6,16–19]

Two major subgroups of euglenids have independently descended from eukaryovorous ancestors and have secondarily lost or reduced the feeding apparatus: (1) primary osmotrophic euglenids and (2) photosynthetic euglenids (Fig. 2). Osmotrophic euglenids lack a feeding apparatus and are able to absorb molecules directly from eutrophic environments (e.g. *Distigma* and *Rhabdomonas*; Fig. 1H–I). The particular set of ultrastructural features found in some osmotrophic euglenids reflects this major switch in nutritional mode (e.g. secondarily fused strips and cell rigidity).[6,10,26,27] Along these lines, many poorly understood euglenids (e.g. *Calkinsia*) thrive in anaerobic environments and have developed ultrastructural features that correspond to tight associations with ectosymbiotic bacteria.[28] Photosynthetic euglenids are a clade of chimeric cells that are derived from a relatively recent secondary endosymbiosis with green algal prey cells (Figs 1J–R, 2—position 6).[4] Although alternative scenarios for plastid origins (e.g. the "plastids early" hypothesis) have been proposed, they have been addressed at length elsewhere and have been shown to be inconsistent with available morphological and molecular phylogenetic evidence.[4] Nonetheless, the nearest sister species to photosynthetic euglenids are eukaryovorous euglenids, like *Peranema* (Figs 1G, 2), that have retained features of the ancestral cell that originally acquired the secondary endosymbiotic chloroplasts.[4,6,17] Although the cytoskeletal features found in the earliest diverging photosynthetic euglenids (e.g. Eutreptiales and some *Euglena*, Fig. 1J) are remarkably similar to the cytoskeletal features found in many eukaryovores, modifications of the cell structure in many photosynthetic euglenids reflect the switch from a phagotrophic to a photosynthetic mode of nutrition (Fig. 1L–R). For instance, photosynthetic euglenids have abandoned gliding motility for swimming motility and employ fundamentally different flagellar beat patterns (e.g. a

lasso or figure-eight beat pattern that pulls the cell forward), allowing them to exploit the water column above the substrate (Fig. 2—position 7).[4]

The superficial cytoskeleton, or "pellicle", in several photosynthetic genera has become increasingly more robust and rigid (e.g. *Monomorphina, Phacus* and *Lepocinclis*; Figs 1L, O–R, 2—position 9), and some photosynthetic genera have developed either hardened shell-like "loricas" (e.g. the loricates, *Trachelomonas* and *Stromobomonas*) or sessile colonies held together by branched mucilaginous stalks (e.g. *Colacium*) (Figs. 1M–N, 2—position 8).[26,29] Moreover, photosynthetic euglenids possess an enlarged flagellar pocket, called a "reservoir", a photosensory swelling at the base of the dorsal (syn. emergent) flagellum and a carotenoid-based shading structure involved in phototaxis, called the "stigma" (syn. eyespot) (Fig. 2—position 6). Photosynthetic euglenids have also retained a vestigial feeding apparatus and, like eukaryovores, some photosynthetic and primary osmotrophic species have retained the ability to undergo rapid peristalsis-like deformations in cell shape, known as "metaboly" or "euglenoid movement" (Fig. 1H, J,K).
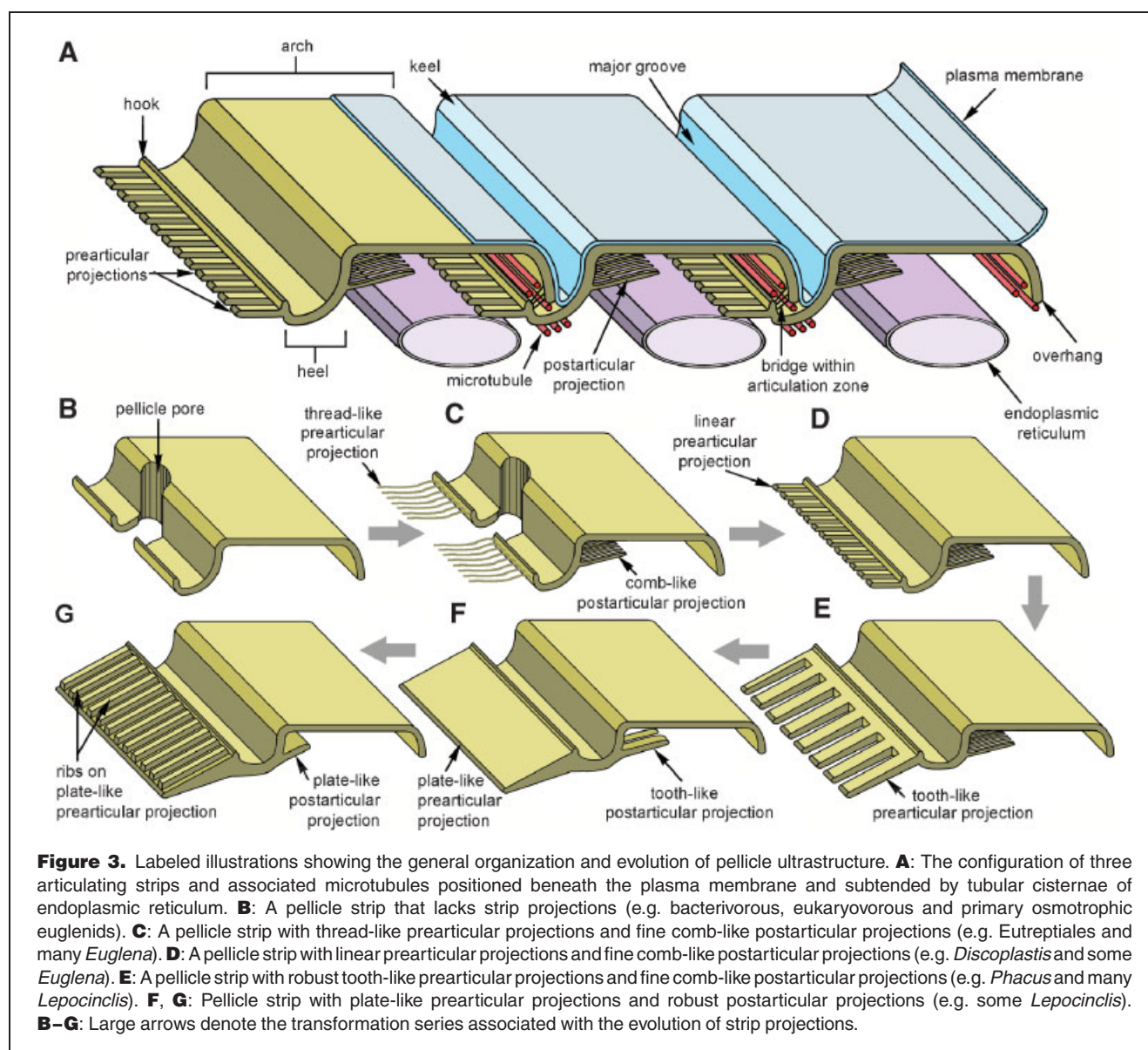
The euglenid cytoskeleton

A network of microtubules supports the flagellar apparatus, feeding apparatus and pellicle of euglenids. Two basal bodies form the core of a microtubular organizing center from which the axonemes of the dorsal and ventral flagella and three associated microtubular roots emerge (Fig. 2—position 1).[4,30–33] Microtubules stemming from two of the roots (i.e. the dorsal root and the intermediate root) reinforce the flagellar pocket and presumably the pellicle. Microtubules stemming from the third root (i.e. the ventral root) reinforce and become structural components of the feeding apparatus.[34–39] The precise manner in which the microtubules of the euglenid cytoskeleton remain integrated throughout cytokinesis is still largely unknown. However, current evidence indicates that the basal bodies and feeding apparatus duplicate early prior to cytokinesis, and the basal bodies segregate into daughter cells in a semi-conservative fashion: each daughter cell receives one of the parent basal bodies and one of the nascent basal bodies.[32,35,37,38] The nascent basal bodies support dorsal flagella in the daughter cells; the parent ventral basal body is maintained and supports a ventral flagellum in a daughter cell; the parent dorsal body is transformed into a ventral basal body before being segregated with a nascent dorsal basal body in the other daughter cell. The feeding apparatus duplicates in coordination with the morphological transformation of the parent dorsal flagellum into a ventral flagellum. Therefore, two rounds of cytokinesis are required before a ventral basal body and the associated ventral flagellum and feeding apparatus are formed and maintained throughout all subsequent cell divisions.[32,40]

Trends in the evolution of pellicle ultrastructure

The euglenid pellicle is a novel system of microtubules, proteinaceous strips and endoplasmic reticulum (ER) that subtends the plasma membrane and runs along the length of the cell (Figs 1, 2—position 2,3A). The strips are either helically twisted (e.g. plastic eukaryovores, plastic osmotrophs and both plastic and rigid photosynthetic euglenids; Fig. 1E–H, J–O, Q–R) or arranged in linear, longitudinal rows (e.g. bacterivores, rigid osmotrophs and some rigid photosynthetic euglenids; Fig. 1A–D, I, P). Helically arranged strips are associated with cell plasticity (metaboly), which is facilitated by relative translational movements between adjacent strips (Fig. 2—position 4).[41–45] The underlying mechanism for euglenoid movement, however, is not well understood. Although experiments have shown that the pellicle microtubules are controlled by calcium stored in the ER and play an important role in changing cell shape,[46] one of the major gaps in knowledge has to do with the precise distribution and morphogenesis of the pellicle microtubules during cytokinesis.

By contrast, the general ultrastructure of proteinaceous strips is relatively well understood. The strips are basically "S-shaped" in transverse section and interlock with adjacent strips along their longitudinal margins, in an area called the "articulation zone" (Fig. 3A). Articulation zones consist of inconspicuous "bridges" that connect the "overhang" of one strip with the "hook" of an adjacent strip (Fig. 3A).[26,47,48] The main region of pellicle strips that is most visible on the cell

Figure 3. Labeled illustrations showing the general organization and evolution of pellicle ultrastructure. **A**: The configuration of three articulating strips and associated microtubules positioned beneath the plasma membrane and subtended by tubular cisternae of endoplasmic reticulum. **B**: A pellicle strip that lacks strip projections (e.g. bacterivorous, eukaryovorous and primary osmotrophic euglenids). **C**: A pellicle strip with thread-like prearticular projections and fine comb-like postarticular projections (e.g. Eutreptiales and many *Euglena*). **D**: A pellicle strip with linear prearticular projections and fine comb-like postarticular projections (e.g. *Discoplastis* and some *Euglena*). **E**: A pellicle strip with robust tooth-like prearticular projections and fine comb-like postarticular projections (e.g. *Phacus* and many *Lepocinclis*). **F**, **G**: Pellicle strip with plate-like prearticular projections and robust postarticular projections (e.g. some *Lepocinclis*). **B–G**: Large arrows denote the transformation series associated with the evolution of strip projections.

surface is the "arch". The (major) grooves between strips are defined by the "heels" of each strip, which in many photosynthetic species are disrupted by "pellicle pores" (Figs 1K, 3A,B), and anchor strip "projections" that interlock with other projections stemming from adjacent strips (Fig. 3A). Strip projections that extend beneath the arch of an adjacent strip are called "prearticular" projections, and strip projections that are positioned beneath the arch of the same strip are called "postarticular" projections (Fig. 3). Although the ultrastructural diversity of pellicle strips in different species reflects phylogenetic relationships and is correlated with different modes of nutrition, compelling explanations for the adaptive significance of this diversity are not at all obvious.

Nevertheless, detailed comparative analyses within a molecular phylogenetic framework have demonstrated some persuasive trends in the evolution of strip ultrastructure (Fig. 3). For instance, all phagotrophic and primary osmotrophic euglenids examined so far lack strip projections (Fig. 3B), whereas all photosynthetic euglenids possess them.[5,6,26] The strip projections observed in the earliest diverging photosynthetic euglenids (e.g. Eutreptiales) are inconspicuously fine and referred to as "thread-like" prearticular projections and "comb-like" postarticular projections (Figs 2—position 6, 3C). These types of strip projections have also been described in other photosynthetic genera, such as *Colacium* (Fig. 1M), *Trachelomonas* (Fig. 1N) and several members of *Euglena* (e.g. *E. mutabilis*, Fig. 1J). The width and thickness of the proteinaceous strips themselves (the arches and heels) are also relatively narrow and thin in these genera. Moreover, dynamic patterns of euglenoid movement are correlated with pellicles consisting of many helical strips (>20) that either have fine, thread-like strip projections (e.g. *Eutreptia* and *Euglena*, Fig. 1J–K) or lack projections altogether (e.g. *Peranema* and *Distigma*, Fig. 1G–H).

A gradual increase in the robustness and size of pellicle strips, including the strip projections, has been well demonstrated and is correlated with decreases in cell plasticity (Figs 2—position 9, 3B–G).[5,26,41,47,49] More robust, "linear" prearticular projections are found in *Discoplastis* and several species of weakly metabolic *Euglena,* and even thicker, "tooth-like" strip projections are found in (relatively) rigid photosynthetic genera, namely *Phacus, Lepocinclis* and *Monomorphina* (Figs 2, 3). Within *Lepocinclis*, robust "plate-like" strip projections and enormous strips have evolved from tooth-like precursors in association with dramatic increases in overall cell size (e.g. *L. helicoideus, L. oxyuris,* and *L. spirogyra*; Figs 1R, 2, 3F–G).[26,42,44,50] The evolutionary transformation from fine, thread-like projections to robust tooth-like (or plate-like) projections within the photosynthetic euglenids has occurred independently at least three times: the *Monomorphina-Cryptoglena* clade, the *Phacus* clade and the *Lepocinclis* clade (Fig. 2). However, the most-recent common ancestor of *Phacus* and *Lepocinclis* probably already had relatively robust strips, and projections, that were similar to those found in *Discoplastis*.

Independent increases in strip size and robustness within photosynthetic euglenids is inferred to be an evolutionary response to the secondary endosymbiotic origin of chloroplasts and the associated nutritional switch from phagotrophy to photosynthesis (Fig. 2—position 6).[4] As argued previously, plastic pellicles (metaboly) facilitate eukaryovorous modes of feeding, because distension of the predatory cell can accommodate the ingestion of large prey cells (e.g. diatoms). It is very clear that plastic pellicles and eukaryovory evolved prior to the secondary endosymbiotic event, and metaboly in photosynthetic euglenids and primary osmotrophs might simply be a vestige of a eukaryovorous ancestry. This example of morphostasis is completely consistent with molecular phylogenetic data, in that the most plastic photosynthetic euglenids (Eutreptiales) diverge earliest among other photosynthetic species and the nearest sister lineage to the photosynthetic euglenids are highly plastic eukaryovores (Fig. 2—position 5). Nonetheless, although the loss of cell plasticity in several photosynthetic lineages is consistent with the secondary loss of eukaryovory, the adaptive causation associated with the convergent evolution of robust strips (including the projections) remains elusive and, at best, a matter of speculation.

Evolution and development of pellicle surface patterns

Photosynthetic euglenids also possess novel pellicle surface patterns that are enigmatic from the perspective of functional morphology, but are extremely insightful in understanding developmental mechanisms involved in the evolutionary diversification of the group.[5,8,27,29,50] These surface patterns are a manifestation of underlying developmental processes that probably have little, if anything, to do with adaptive fitness (i.e. so-called "exaptations"). Pellicle surface patterns exist at both ends of euglenid cells but are most conspicuous at the posterior end, where some pellicle strips are shorter than their immediate neighboring strips and form circular or "whorled" patterns of strip reduction (Figs 1K, 4). The number of whorls of *posterior* strip reduction is consistent within species, but usually varies between species (Fig. 4). However, patterns of strip reduction are occasionally consistent within genera (or other more inclusive clades), such as the loricate genera *Trachelomonas* and *Strombomonas*, which have one and two posterior whorls of reduction, respectively.[27,29] While whorled patterns of strip reduction are not directly linked to any other discernable pellicle character state, such as the shape of the posterior tip,[5] whorled patterns are undoubtedly the products of differential strip growth during cell division.[7]

Just prior to cell division, the pellicle duplicates by doubling the number of strips. Nascent pellicle strips emerge between existing parent strips near the opening of the flagellar pocket and simultaneously migrate posteriorly over the cell surface
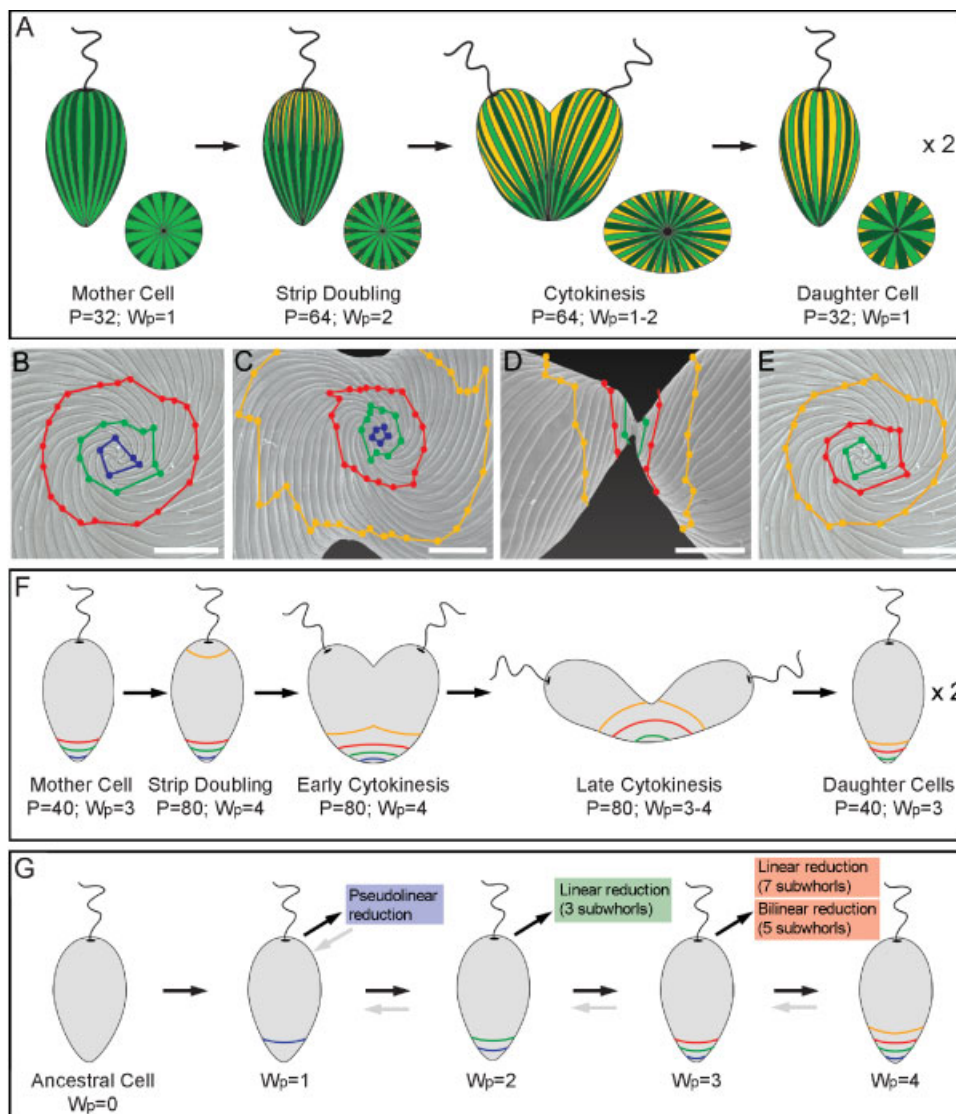
Figure 4. Morphogenesis, inheritance and evolution of pellicle surface patterns called "whorls of posterior strip reduction". P, the total number of strips around the cell periphery; Wp, number of posterior whorls of strip reduction. **A**: An illustration showing the general model of pellicle replication during cytokinesis. The parent cell contains one whorl of posterior strip reduction (dark green) resulting from every other strip on the cell terminating before reaching the posterior tip; strips that extend to the posterior tip of the cell are indicated by light green. Just prior to cell division, nascent strips (yellow) emerge between each parent strip (green) and grow toward the posterior end of the cell. The nascent strips terminate before reaching the posterior tip and become a new whorl of strip reduction in each daughter cell. The strips that formed the whorl of strip reduction in the parent cell (dark green) grow toward the posterior end prior to cytokinesis and converge with the light green strips at the posterior tip of the daughter cells. **B−E**: Scanning electron micrographs of *Euglena gracilis* showing the development of three whorls of posterior strip reduction during cytokinesis. **F**: A simpler illustration of the patterns of strip growth shown in B−E. **G**: Illustrations showing the known diversity in pellicle surface patterns. Arrows denote the hypothetical transformation series associated with the evolution of Wp. See main text for discussion. Figure modified from Esson HJ, Leander BS 2006 Evol Develop 8:378−388.

and into the flagellar pocket (Fig. 4A).[51,52] Cytokinesis then proceeds longitudinally from the anterior end of the cell to the posterior end, where each daughter cell inherits an equal number of nascent strips intercalated between parent strips (Fig. 4A). Most photosynthetic euglenids possess two or more whorls of posterior strip reduction, which provide additional insight into the multigenerational origins of individual pellicle strips. For instance, *Euglena gracilis* possesses three whorls of exponential strip reduction. As cytokinesis progresses in this species, growing nascent strips (indicated with yellow),

extend toward the posterior end of the cell and eventually occupy the same position as the first whorl in the parent cell (indicated with red, Fig. 4C–F). The strips that formed each of the three parental whorls extend downward during cytokinesis and occupy the position of the next whorl in the series, respectively (Fig. 4B–F). The strips that formed the last whorl in the parent cell (indicated with blue) merge with the strips that were already present at the posterior tip of the parent cell. This dynamic process maintains multi-whorled patterns of strip reduction from generation to generation, and each whorl in both the parent and daughter cells reflects different episodes of strip duplication (Fig. 4B–F).[7] The pellicle of *E. gracilis*, therefore, consists of more than four different generations of strips at any given time, with three generations comprising the three whorls of posterior reduction, and more than two generations comprising the strips that reach the posterior tip of the cell (Fig. 4A–F).[7]

This multigenerational pattern of pellicle strip inheritance suggests that modifications in the timing of developmental processes—i.e. "heterochrony"—played an important role in the diversification and evolution of the euglenid cytoskeleton. The range of character states observed for posterior strip reduction in euglenids strongly indicates that the ancestral condition was the absence of whorls altogether, and cells with many whorls of strip reduction (e.g. four in *Euglena rustica*) evolved from ancestors with fewer whorls of strip reduction along a transformation series of intermediate states (Figs 1, 2, 4G). The origin of one whorl of strip reduction is inferred to be the outcome of an ancestral (photosynthetic) cell with nascent strips that failed to grow to full length during cytokinesis. The serial repetition of this phenomenon explains the presence of two, three and four whorls of strip reduction in other euglenid cells (Fig. 4G). Moreover, differentiation in the length of strips within one or more whorls of exponential strip reduction has produced other novel surface patterns that have been described in several species of *Euglena* and *Lepocinclis* (e.g. "linear" and "bilinear" patterns, Fig. 4G).[5,8,27,50] Although beyond the scope of this essay, these patterns indicate that the relative age of parent strips plays an important role in the developmental patterning of nascent strips.[8] This phenomenon is reminiscent of the epigenetic patterns of inheritance prominently described in *Paramecium* by Tracy Sonneborn's research group.

Origin, diversification and adaptive significance of pellicle strip number

The earliest stages in the evolution of the euglenid pellicle, especially the proteinaceous strips, are not well understood. However, we can confidently infer that the most-recent euglenid ancestor had a thin proteinaceous layer of some kind—the "proto-strip"—that subtended the plasma membrane. Moreover, a broad comparison of the extant diversity in pellicle ultrastructure, coupled with the developmental processes outlined above, provides some compelling insights into how this ancestral proteinaceous layer subsequently diversified into many, articulating strips that eventually afforded a mechanism for dynamic euglenoid movements. The total number of pellicle strips around the periphery of a euglenid cell, abbreviated "P", is generally consistent within species and varies considerably between species.[5,27]

The upper, gray portion of Fig. 5 provides a hypothetical scenario for the initial development of pellicle strips. As inferred from the structure of extant euglenids and their nearest sister groups (kinetoplastids and diplonemids, Fig. 2), an ancestral bacterivore that completely lacked a proteinaceous pellicle ($P = 0$) must have given rise to similar cells with a thin proto-strip beneath the plasma membrane ($P = 1$; indicated by "purple"). The adaptive significance for the initial proteinaceous layer remains speculative, but it certainly enhanced the structural integrity of the cell and might be related to changes in gliding capabilities and cell–substrate interactions. Although the articulation zones between the strips of extant euglenids would be absent in this hypothetical ancestor, known patterns of intussusceptive pellicle strip duplication and development indicate that protein deposition prior to cell division would have been localized in a similar manner.[7,51,52] Therefore, we hypothesize that a longitudinal zone on the cell surface, termed the "proto-articulation" (Fig. 5), was the site of protein deposition during pellicle duplication.

This proto-articulation zone is also important for conceptualizing the position of the cleavage furrow, which would have formed on either side of the newly deposited protein layer (indicated by "white" in the gray portion of Fig. 5). At this early stage in the evolution of euglenids, the proto-articulation would not only allow for localized protein deposition and the maintenance of one proto-strip in each daughter cell, but would also result in daughter cells with proto-strips of different ages. Accordingly, the underlying cytoskeletal microtubules would still be duplicated and inherited in the same semi-conservative manner that was described previously for the basal bodies and the associated flagella and root system.[40,53,54] Interestingly, in all phagotrophic euglenids described so far, the ventral flagellum (if present) is tightly integrated with one specific ventral strip, the so-called "flagellar strip", that is morphologically distinct from the other strips around the cell (Figs 1, 5). It is possible that the flagellar strips in extant species either reflect, or are in fact directly descended from, the proto-strips of the earliest euglenid ancestors. Because our current knowledge of euglenozoan diversity is still poor, the possible discovery of extant species that have retained the ancestral characters described above is well within reasonable expectations; thus, the hypothesis outlined in the gray portion of Fig. 5 can be tested or refined with improved knowledge of euglenozoan diversity.
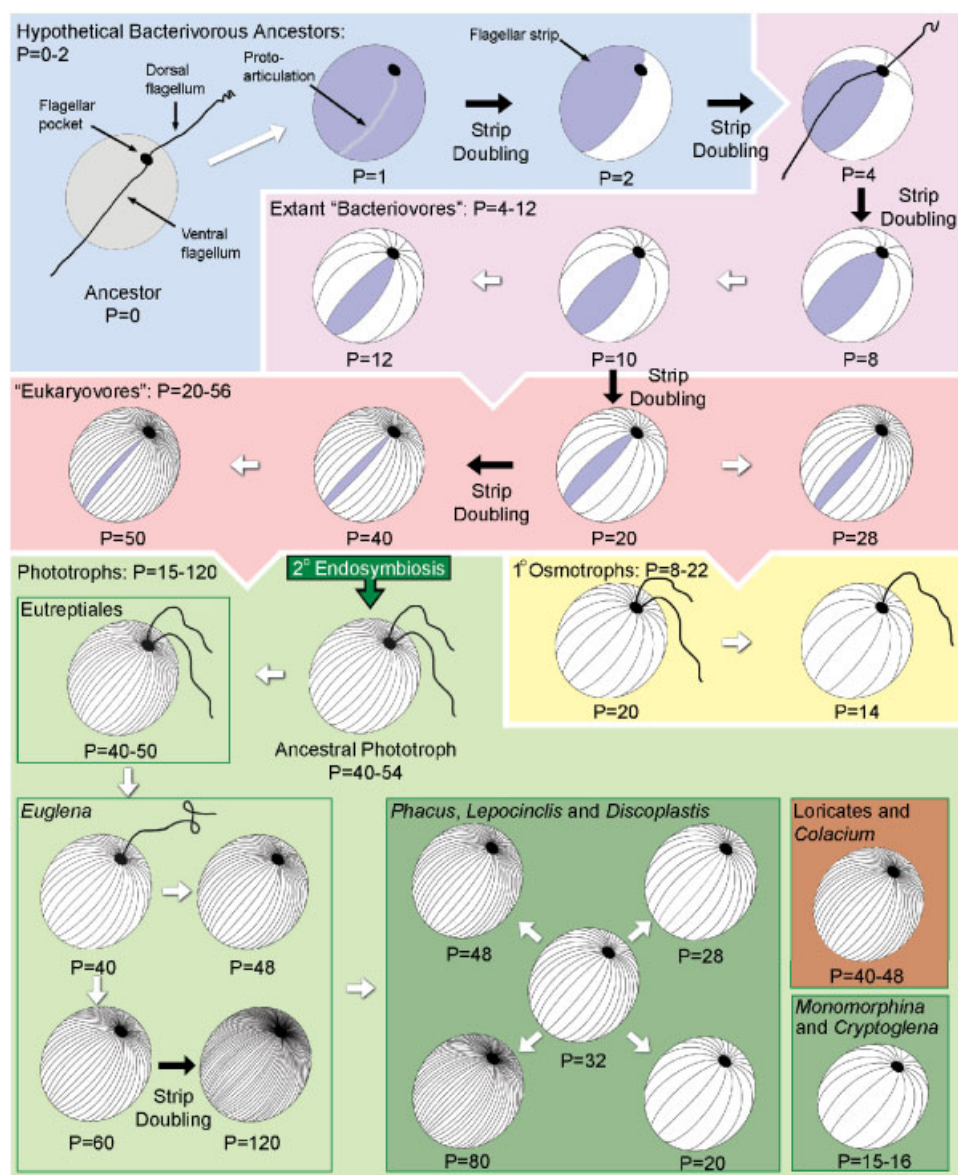
Figure 5. Evolution of the total number of pellicle strip around the cell periphery, abbreviated "P". Gray background contains hypothetical bacterivorous ancestors; light pink background contains known states for P in extant bacterivorous; dark pink background contains known states for P in extant eukaryovores; yellow background contains known states for P in extant primary osmotrophes; light green background contains known states for P in extant photosynthetic eugelenids with plastic pellicles; dark green background contains known states for P in extant photosynthetic euglenids with rigid or semi-rigid pellicles; dark orange background contains known states for P in extant photosynthetic euglenids either encased in a lorica or connected by mucilagenous stalks. Large arrows denote the hypothetical transformation series associated with the evolution of P. See main text for discussion.

In order to better understand the origins and subsequent evolution of the euglenid pellicle, a great deal of research is required in the following areas: (1) the diversity and phylogeny of phagotrophic euglenids and related heterotrophic flagellates and (2) developmental processes (e.g. genetics and live-cell imaging) in euglenids, kinetoplastids and related taxa.

Nonetheless, comparative analyses focused on the number of pellicle strips in extant species indicate that the value of P is strongly correlated with modes of nutrition (Fig. 5). Bacterivorous euglenids, for instance, have few longitudinally arranged strips ($P = 4-12$) and relatively rigid cells. Eukaryovorous euglenids have many, helically arranged strips

($P = 20-56$) and relatively plastic cells (Fig. 5). This combination of pellicle characters enables eukaryovorous euglenids to hunt and engulf large prey cells, such as the green algal prey that ultimately gave rise to the secondary plastids in photosynthetic euglenids (Figs 2—position 6, 5).[4]

Euglenid species derived from ancestors that lost eukaryovorous modes of nutrition show general evolutionary trends toward the loss of cell plasticity and a decrease in strip number (Figs 1, 2, 5). For instance, primary osmotrophs include plastic species, such as *Distigma* with $P = 18-22$, and more derived rigid species, such as *Rhabdomonas* and *Menoidium* with $P = 8-14$. Species of photosynthetic euglenids, which have replaced eukaryovory with photosynthesis, possess a wide range of values for P that reflects different phylogenetic positions, cell sizes and relative cell rigidity (Figs 1, 2, 5). Photosynthetic species with plastic pellicles, such as the early-diverging Eutreptiales as well as *Euglena, Colacium* and the loricate genera *Trachelomonas* and *Strombomonas*, have strip numbers that are similar to those in several extant eukaryovores (e.g. *Peranema* and *Urceolus*) and the inferred photosynthetic ancestor ($P = 40-50$; Figs 1, 2, 5).[27,29] Some highly plastic species of *Euglena* thrive in interstitial environments and possess the largest number of strips known (e.g. *E. obtusa*, $P = 120$).[8] By contrast, photosynthetic species with rigid pellicles, namely *Phacus, Lepocinclis, Cryptoglena* and *Monomorphina*, show independent trends toward fewer strips; some species of *Phacus* have 20 strips, and species in *Cryptoglena* and *Monomorphina* have 15 and16 strips, respectively (Figs 1, 5).[5,27,49] Some species of *Lepocinclis*, however, have extremely large P values that are correlated with significant increases in cell size (e.g. *L. helicoideus*, $P = 80$).[50]

Patterns of pellicle strip development provide important clues into the underlying mechanism(s) associated with the evolutionary diversification of strip number. As described previously, when a euglenid cell prepares for division, a new pellicle strip develops between every pair of parent strips, which doubles the number of strips in the pre-divisional cell. The cleavage furrow forms between a nascent strip and a parent strip on opposite sides of the canal opening, such that half of the strips form the pellicle of each daughter cell (Fig. 4).[7,54] Differences in the number of strips in extant species indicate that, at several points in pellicle evolution, strip duplication was not coupled with cell division, which resulted in one "composite daughter" cell with twice as many strips as before.[5,6] These strip-doubling events are inferred to have taken place several times in euglenid evolution: (1) during the early evolution of pellicle strips in bacterivores ($P = 2 \rightarrow 4$; $P = 4 \rightarrow 8$), (2) in the transition from bacterivorous modes of nutrition to eukaryovorous modes of nutrition ($P = 10 \rightarrow 20$), (3) within eukaryovorous ($P = 20 \rightarrow 40$), and (4) within photosynthetic euglenids (e.g. $P = 60 \rightarrow 120$) (Figs 2, 5). Because P does not always vary by discrete intervals between species and minor variation in P has been observed within species,

mechanisms other than strip-doubling must be responsible for some of the observed diversity in strip number. This variation is likely caused by misplacement of the cleavage furrow during division, resulting in anomalous or uneven segregation of pellicle strips in the daughter cells.[7] Moreover, strip-doubling events appear to be autonomous from the replication and evolution of another major cytoskeletal system linked to the flagellar basal bodies, namely the euglenid feeding apparatus.

Evolutionary morphology of the euglenid feeding apparatus

Phagotrophic euglenids possess a distinctive feeding apparatus for the capture and ingestion of prey cells. Although the overall structural and functional diversity of the euglenid feeding apparatus is poorly known, it is clear that some euglenids have relatively simple feeding systems and others have exceedingly complex ones (Fig. 6). Previous authors proposed that different feeding systems constitute a transformation series, ranging from a relatively simple and ancestral "type I" apparatus to a more-complex and derived "type IV" apparatus (Fig. 6G).[24,25] However, hypotheses for trends in the evolution of the euglenid feeding apparatus are crippled by the meagre sample of phagotrophic species that have been investigated so far. Nonetheless, a brief review of what is currently known about euglenid feeding systems not only helps highlight some putative evolutionary trends, but also emphasizes some major gaps in our knowledge.

Type I feeding systems—or "microtubule reinforced (MTR) pockets"—are present in small bacterivores, such as *Petalomonas* and *Calycimonas* (plus some kinetoplastids), and consist of a ventral invagination that is reinforced by microtubules stemming from the ventral root of the ventral basal body (Figs 1, 6G).[24,25,55,56] The anterior-most part of the feeding pocket is continuous with the flagellar pocket, forming a single opening near the anterior end of the cell. The MTR pockets of euglenids are very similar and homologous to the feeding systems in their nearest relatives, namely bacterivorous kinetoplastids (i.e. bodonids) (Fig. 2—position 1).[24,25,56]

The feeding systems in most phagotrophic euglenids, however, comprise an integrated system of proteinaceous "rods" and "vanes" (Figs 2—position 3, 6). For instance, the feeding apparatus in the bacterivore *Ploeotia* consists of robust rods that extend the entire length of the cell and are reinforced by a superficial layer of microtubules and an internal amorphous matrix (Figs 1C, 6A, G).[25,31,38,57] The feeding rods found in the bacterivore *Entosiphon* are the most intricate of all known euglenids. The rods in these predators lack an amorphous matrix and, instead, are completely reinforced with an elaborate array of microtubules. In addition, one of the supporting rods also bifurcates near the posterior end of the cell, giving rise to three rods that extend the length of the cell (Figs 1D, 6B, G).[37,38,40,58] The feeding apparatus in *Entosiphon* also has a distinctive and continuous behavior associated
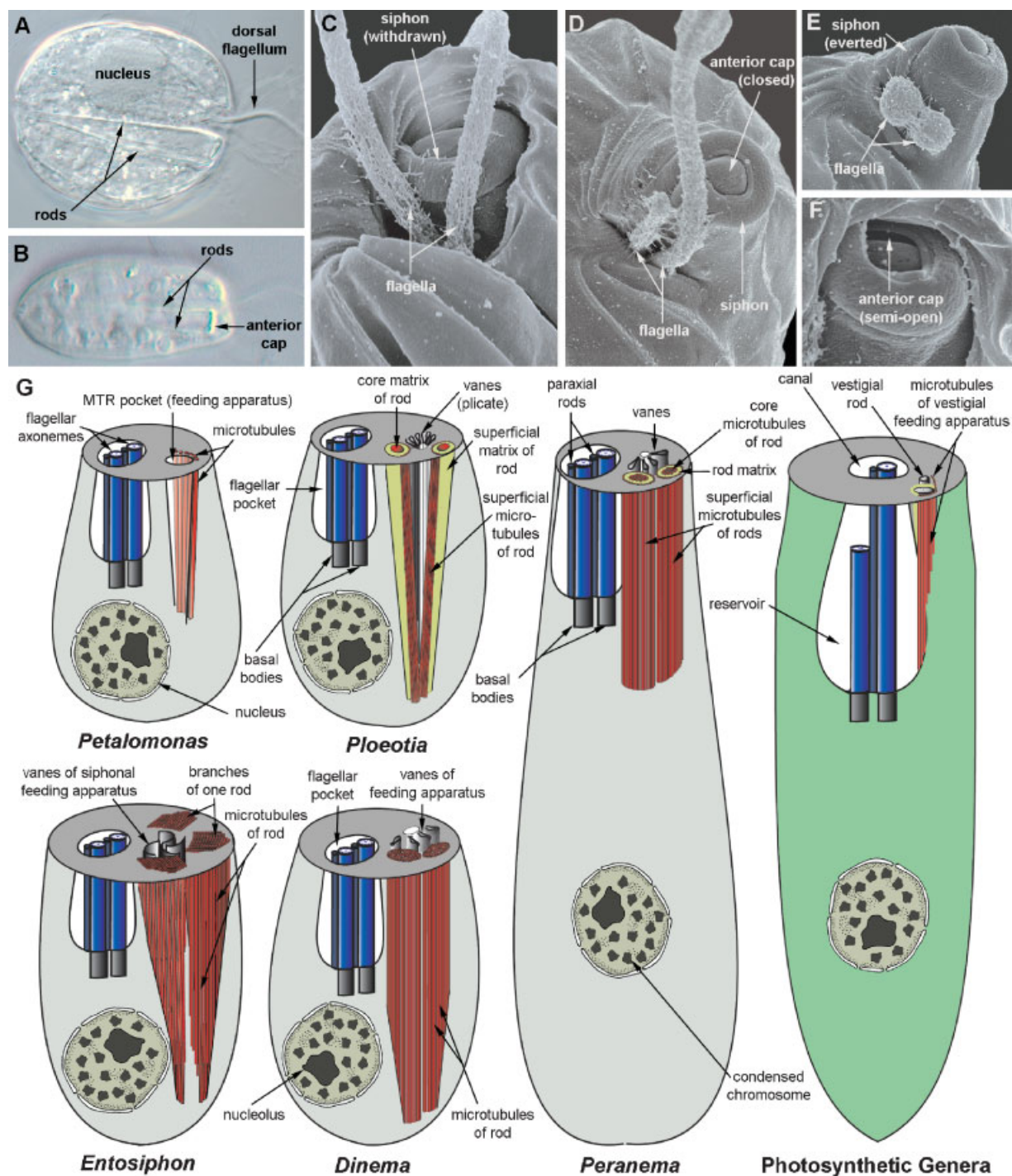
Figure 6. Comparative morphology of the euglenid feeding apparatus. **A**: Light micrograph showing the longitudinal orientation of the feeding rods in *Ploeotia* sp. **B**: Light micrograph showing the anterior cap and longitudinal orientation of the feeding rods in *Entosiphon sulcatum*. **C–F**: Scanning electron micrographs of the feeding apparatus (or siphon) in *Entosiphon sulcatum*. **G**: Labeled diagrams showing the general organization and known diversity of the euglenid feeding apparatus. For illustrative purposes, the anterior end of each cell is shown in transverse section. Red, microtubules; yellow, amorphous matrix; blue, flagellar axonemes. See main text for discussion.

with the pronounced extension of the rods out from the anterior end of the cell (Fig. 6C–F). This so-called "siphon" is covered by an "anterior cap" that only opens when the siphon is fully extended in order to allow prey cells to be drawn into the cell (Fig. 6B–F).[37,38,40,58] Extension of the siphon also simultaneously closes off the opening to the flagellar pocket (Fig. 6D–E). The high degree of complexity associated with these feeding systems is correlated with rigid pellicles, which generally limits these predators to bacteria-size prey organisms.

The feeding systems described for eukaryovorous species consist of an elaborate cytostome surrounded by four vanes and two rods that are either entirely reinforced by microtubules (e.g. *Dinema*) or partially reinforced by microtubules embedded in an amorphous matrix (e.g. *Peranema*) (Fig. 6G).[24,33,59] The relative density of microtubules and the amorphous matrix surrounding them appears to vary considerably in the rods of different species. Like in bacterivorous euglenids, the rods in some eukaryovores extend the entire length of the cell, and this configuration is present in eukaryovorous euglenids with approximately 20 pellicle strips that are only slightly helically arranged, such as *Dinema* (Figs 1E–F, 6G). This set of character states is inferred to reflect the transition from a predominantly bacterivorous mode of nutrition to a eukaryovorous one (Fig. 2—position 4).[6] Eukaryovorous euglenids with approximately 40–50 strips that are strongly helically arranged (and highly plastic) contain feeding rods that are limited to the anterior third of the cell (Figs 1G, 2—position 5, 6G). This set of character states is a derived condition associated with a greater capacity for acquiring and consuming eukaryotic prey cells.[4,5,60] For instance, a reduction of the rod length eliminates structural constraints in the posterior two thirds of the cell, which presumably gives these euglenids the flexibility to simultaneously accommodate the ingestion of several large prey cells.

Vestiges of a feeding apparatus are present in photosynthetic euglenids as well, which reflects the phagotrophic ancestry of this lineage and substantiates the inferred secondary endosymbiotic origin of the chloroplasts (Fig. 6G).[34,35,39,56,61–62] The relict feeding systems in photosynthetic euglenids consist of a cytoplasmic pocket, reinforced by a few microtubules, that is superficially similar in structure and position to the simple MTR pockets present in petalomonad euglenids (Figs 2—position 6, 6G). Although several alternative hypotheses have been proposed (including the suggestion that two different feeding systems might exist within one *Dinema*-like cell), evidence from comparative morphology and molecular phylogenetics strongly indicates that the feeding apparatus in photosynthetic euglenids is derived most directly from the general feeding systems found in eukaryovores euglenids, such as *Peranema* and *Urceolus*.[4–6,36] Nonetheless, the overall evolutionary history of the euglenid feeding apparatus is far from complete, and major areas of uncertainty include the origin and early evolution of the rods and vanes from MTR pockets and the extent of the subsequent diversification.

Conclusions

Microbes are often assumed to be deficient in morphological diversity. Although this perspective might be accurate for most prokaryotes, it is categorically false for the vast majority of eukaryotic microbes and their multicellular descendants: animals, fungi and land plants. Unlike prokaryotes, the cells of eukaryotes cannot be considered truly unicellular, because they are at minimum a nested set of at least two previously independent cells formed by an endosymbiotic merger (i.e. a mitochondrion within a nucleated host cell). Furthermore, many diverse groups of eukaryotes are the result of additional mergers involving the endosymbiotic acquisition of either primary or secondary plastids. These endosymbiotic events caused punctuated changes in nutritional mode (phagotrophy → photosynthesis) that significantly impacted the subsequent evolution of other cellular traits, especially cytoskeletal systems involved in locomotion and feeding. By possessing complex character combinations resulting from adaptive change and morphostasis, different lineages of eukaryotic microbes have retained key pieces of evidence necessary for reconstructing major events in the evolution and diversification of eukaryotic life. Moreover, the vast morphological diversity found in microscopic eukaryotes suggests that, in order to fully comprehend the genetic underpinnings of organismal form and function, the science of developmental biology should dedicate more attention to the ultrastructural systems found in these lineages.

Euglenids represent an excellent example of how understanding patterns of extant morphological diversity provides compelling insights into developmental processes and evolutionary transformations of morphological characters. Evidence strongly suggests that eukaryovorous euglenids descended from bacterivorous ancestors following an increase in the number and helical arrangement of pellicle strips, which conferred greater cell plasticity. Following the secondary endosymbiotic origin of chloroplasts, the pellicle strips became more tightly articulated through a system of overhangs, bridges, hooks and strip projections. The transformation of delicate strip projections into more robust projections was concomitant with increases in cell rigidity in several different photosynthetic lineages. Photosynthetic euglenids also possess pellicle surface patterns, or whorls of posterior strip reduction, that reflect changes in developmental programs during the evolutionary history of the group. The complexity of pellicle surface patterns is best understood within the context of a transformation series built from intermediate surface patterns described in different species.

During the course of euglenid evolution, the number of pellicle strips steadily increased from very few ($P = 2$–4) to over one hundred ($P = 120$), and this general trend was

punctuated by permanent strip duplication events resulting from mutations in developmental programs. However, the overall pattern of euglenid diversification demonstrates that strip number has also decreased several times independently in association with fundamental switches in the mode of nutrition (i.e. eukaryovory → osmotrophy and eukaryovory → photosynthesis). Switches in the mode of nutrition are also reflected in the morphology of the euglenid feeding apparatus. An ancestral MTR pocket gave rise to feeding rods and vanes that extend the entire length of the cell and are elaborately reinforced with microtubules in many bacterivores and early diverging eukaryovores. The length of the feeding rods and vanes is significantly reduced in more-derived eukaryovores and only vestiges of the feeding apparatus remain in photosynthetic euglenids. These patterns of diversification help substantiate the perspective that reductive evolution is to be expected within groups of organisms that have moved sufficiently far away from a (metaphorical) "wall" of minimum complexity.

Our understanding of the phylogenetic relationships and ultrastructural diversity within phagotrophic euglenids, in particular, is still in its infancy. Although we are beginning to notice the developmental processes controlling the diversity of euglenid morphology, we have yet to even scratch the surface in trying to understand the genetic and epigenetic foundations for cell differentiation in the group. This understanding could shed considerable light onto underlying processes of cell differentiation that are shared by all eukaryotes or perhaps shared only between members of specific supergroups. From this perspective, it is a very exciting time to be a comparative cell biologist, because like euglenids, essentially every major group of microbial eukaryotes provides a large set of open questions that we are only just beginning to explore.

Acknowledgments

References

1. Embly TM, Martin W. 2006. Eukaryotic evolution, changes and challenges. Nature 440:623–630.
2. Archibald JM. 2005. Jumping genes and shrinking genomes—probing the evolution of eukaryotic photosynthesis with genomics. IUBMB Life 57:539–547.
3. Gilson PR, Su V, Slamovits CH, Reith ME, Keeling PJ, McFadden GI. 2006. Complete nucleotide sequence of the chlorarachniophyte nucleomorph: Nature's smallest nucleus. Proc Nat Acad Sci USA 103:9566–9571.
4. Leander BS. 2004. Did trypanosomatid parasites have photosynthetic ancestors? Trends Microbiol 12:251–258.
5. Leander BS, Witek R, Farmer MA. 2001. Trends in the evoluton of the euglenid pellicle. Evolution 55:2115–2135.
6. Leander BS, Triemer RE, Farmer MA. 2001. Character evolution in heterotrophic euglenids. Europ J Protistol 37:337–356.
7. Esson HJ, Leander BS. 2006. A model for the morphogenesis of strip reduction patterns in photosynthetic euglenids: Evidence for heterochrony in pellicle evolution. Evol Develop 8:378–388.
8. Esson HJ, Leander BS. 2008. Novel pellicle surface patterns on *Euglena obtusa* Schmitz (Euglenophyta), a euglenophyte from a benthic marine environment: Implications for pellicle development and evolution. J Phycol 44: In press
9. Keeling PJ, Burger G, Durnford DG, Lang BF, Lee RW et al. 2005. The tree of eukaryotes. Trends Ecol Evol 20:670–676.
10. Preisfeld A, Busse I, Klingberg M, Talke S, Ruppel HG. 2001. Phylogenetic position and inter-relationships of the osmotrophic euglenids based on SSU rDNA data, with emphasis on the Rhabdomonadales (Euglenozoa). Int J Syst Evol Microbiol 51:751–758.
11. Busse I, Patterson DJ, Preisfeld A. 2003. Phylogeny of phagotrophic euglenids (Euglenozoa): a molecular approach based on culture material and environmental samples. J Phycol 39:828–836.
12. von der Heyden S, Chao EE, Vickerman K, Cavalier-Smith T. 2004. Ribosomal RNA phylogeny of bodonid and diplonemid flagellates and the evolution of euglenozoa. J Eukaryot Microbiol 51:402–416.
13. Triemer RE, Linton E, Shin W, Nudelman A, Monfils A et al. 2006. Phylogeny of the euglenales based upon combined SSU and LSU rDNA sequence comparisons and description of *Discoplastis* gen. nov (Euglenophyta). J Phycol 42:731–740.
14. Marin B, Palm A, Klingberg M, Melkonian M. 2003. Phylogeny and taxonomic revision of plastid-containing euglenophytes based on ssu rDNA sequence comparisons and synapomorphic signatures in the ssu rRNA secondary structure. Protist 154:99–145.
15. Milanowski R, Kosmala S, Zakrys B, Kwiatowski J. 2006. Phylogeny of photosynthetic euglenophytes based on combined chloroplast and cytoplasmic SSU rDNA sequence analysis. J Phycol 42:721–730.
16. Montegut-Felkner AE, Triemer RE. 1997. Phylogenetic relationships of selected euglenoid genera based on morphological and molecular data. J Phycol 33:512–519.
17. Breglia SA, Slamovits, CH, Leander BS. 2007. Phylogeny of phagotrophic euglenids (Euglenozoa) as inferred from hsp90 gene sequences. J Eukaryot Microbiol 52:86–94.
18. Talke S, Preisfeld A. 2002. Molecular evolution of euglenozoan paraxonemal rod genes *par1* and *par2* coincides with phylogenetic reconstruction based on small subunit rDNA data. J Phycol 38:995–1003.
19. Simpson AGB, Roger AJ. 2004. Protein phylogenies robustly resolve deep-level relationships within Euglenozoa. Mol Phyl Evol 30:201–212.
20. Simpson AGB, Lukes J, Roger AJ. 2002. The evolutionary history of kinetoplastids and their kinetoplasts. Mol Biol Evol 19:2071–2083.
21. Simpson AGB. 2003. Cytoskeletal organisation, phylogenetic affinities and systematics in the contentious taxon Excavata (Eukaryota). Int J Sys Evol Microbiol 53:1759–1777.
22. Simpson AGB, Inagaki Y, Roger AJ. 2006. Comprehensive multi-gene phylogenies of excavate protists reveal the evolutionary positions of 'primitive' eukaryotes. Mol Biol Evol 23:615–625.
23. Saito A, Suetomo Y, Arikawa M, Omura G, Khan SMMK, et al. 2003. Gliding movement in *Peranema trichophorum* is powered by flagellar surface motility. Cell Motil Cytoskel 55:244–253.
24. Triemer RE, Farmer MA. 1991. The ultrastructural organization of the heterotrophic euglenids and its evolutionary implications. In: Patterson DJ, Larsen J editors The Biology of Free-living Heterotrophic Flagellates Clarendon Press. pp 185–204.
25. Triemer RE, Farmer MA. 1991. An ultrastructural comparison of the mitotic apparatus, feeding apparatus, flagellar apparatus and cytoskeleton in euglenoids and kinetoplastids. Protoplasma 164:91–104.
26. Leander BS, Farmer MA. 2001. Comparative morphology of the euglenid pellicle. II. Diversity of strip substructure. J Eukaryot Microbiol 48:202–217.
27. Leander BS, Farmer MA. 2000. Comparative morphology of the euglenid pellicle. I. Patterns of strips and pores. J Eukaryot Microbiol 47:469–479.
28. Bernhard JM, Buck K, Farmer MA, Bowser SS. 2000. The Santa Barbara Basin is a symbiosis oasis. Nature 403:77–80.
29. Brosnan S, Brown PJP, Farmer MA, Triemer RE. 2005. Morphological separation of the euglenoid genera *Trachelomonas* and *Strombomonas* (Euglenophyta) based on lorica development and posterior strip reduction. J Phycol 41:590–605.
30. Simpson AGB 1997. The identity and composition of the Euglenozoa. Archiv Fur Protistenkunde 148:318–328.

31. Solomon JA, Walne PL, Kivic PA. 1987. *Entosiphon sulcatum* (Euglenophyceae): flagellar roots of the basal body complex and reservoir regions). J Phycol 23:85–98.

32. Farmer MA, Triemer RE. 1988. Flagellar systems in the euglenoid flagellates. BioSystems 21:283–291.

33. Hilenski LL, Walne PL. 1985. Ultrastructure of the flagella of the colorless phagotroph *Peranema trichophorum* (Euglenophyceae. II. Flagellar roots). J Phycol 21:125–134.

34. Shin W, Boo SM, Triemer RE. 2001. Ultrastructure of the basal body complex and putative vestigial feeding apparatus in *Phacus pleuronectes* (Euglenophyceae). J Phycol 37:913–921.

35. Surek B, Melkonian M. 1986. A cryptic cytostome is present in *Euglena*. Protoplasma 133:39–49.

36. Shin WG, Brosnan S, Triemer RE. 2002. Are cytoplasmic pockets (MTR/pocket) present in all photosynthetic euglenoid genera? J Phycol 38:790–799.

37. Belhadri A, Brugerolle G. 1992. Morphogenesis of the feeding apparatus of *Entosiphon sulcatum*: an immunofluorescence and ultrastructural study. Protoplasma 168:125–135.

38. Belhadri A, Bayle D, Brugerolle G. 1992. Biochemical and immunological characterization of intermicrotubular cement in the feeding apparatus of phagotrophic euglenoids: *Entosiphon, Peranema*, and *Ploeotia*. Protoplasma 168:113–124.

39. Willey RL, Wibel RG. 1985. A cytostome/cytopharynx in green euglenoid flagellates (Euglenales) and its phylogenetic implications. BioSystems 18:369–376.

40. Brugerolle G. 1992. Flagellar apparatus duplication and partition, flagellar transformation during division in *Entosiphon sulcatum*. BioSystems 28:203–209.

41. Angeler DG, Müllner AN, Schagerl M. 1999. Comparative ultrastructure of the cytoskeleton and nucleus of *Distigma* (Euglenozoa). Europ J Protistol 35:309–318.

42. Suzaki T, Williamson RE. 1985. Euglenoid movement in *Euglena fusca*: evidence for sliding between pellicular strips. Protoplasma 124:137–146.

43. Suzaki T, Williamson RE. 1986. Ultrastructure and sliding of pellicular structures during euglenoid movement in *Astasia longa* Pringsheim (Sarcomastigophora, Euglenida). J Protozool 33:179–184.

44. Suzaki T, Williamson RE. 1986. Pellicular ultrastructure and euglenoid movements in *Euglena ehrenbergii* Klebs and *Euglena oxyuris* Schmarda. J Protozool 33:165–171.

45. Gallo JM, Schrével J. 1982. Euglenoid movement in *Distigma proteus* I. Cortical rotational movement. Biol Cell 44:139–148.

46. Murray JM. 1981. Control of cell shape by calcium in the Euglenophyceae. J Cell Sci 49:99–117.

47. Dragos N, Peterfi LS, Popescu C. 1997. Comparative fine structure of pellicular cytoskeleton in *Euglena* Ehrenberg. Archiv Fur Protistenkunde 148:277–285.

48. Dubreuil RR, Marrs JA, Bouck GB. 1992. The cytoskeleton of euglenoids; cell form, surface motility, and cell replication are based on a membrane skeleton of repeating strips. In: Menzel D editor The cytoskeleton of the algae (Vol. 4) CRC Press, Inc. pp 59–78.

49. Leander BS, Farmer MA. 2001. Evolution of *Phacus* (Euglenophyceae) as inferred from pellicle morphology and SSU rDNA. J Phycol 37:143–159.

50. Leander BS, Farmer MA. 2000. Epibiotic bacteria and a novel pattern of strip reduction on the pellicle of *Euglena helicoideus* (Bernard) Lemmermann. Europ J Protistol 36:405–413.

51. Mignot JP, Brugerolle G, Bricheux G. 1987. Intercalary strip development and dividing cell morphogenesis in the euglenid *Cyclidiopsis acus*. Protoplasma 139:51–65.

52. Hofmann C, Bouck B. 1976. Immunological and structural evidence for patterned intussusceptive surface growth in a unicellular organism. J Cell Biol 69:693–715.

53. Gull K. 1999. The cytoskeleton of trypanosomatid parasites. Annu Rev Microbiol 53:629–655.

54. Bouck GB, Ngo H. 1996. Cortical structure and function in euglenoids with reference to trypanosomes, ciliates, and dinoflagellates. Int Rev Cytol 169:267–318.

55. Kivic PA, Walne PL. 1984. An evaluation of the possible phylogenetic relationship between eugelnophyta and kinetoplastida. Orig Life 13:269–288.

56. Willey RL, Walne PL, Kivic PA. 1988. Phagotrophy and the origins of euglenoid flagellates. CRC Critic Rev Plant Sci 7:303–340.

57. Linton EW, Triemer RE. 1999. Reconstruction of the flagellar apparatus in *Ploeotia costata* (Euglenozoa) and its relationship to other euglenoid feeding apparatuses. J Eukaryot Microbiol 35:313–324.

58. Triemer RE, Fritz L. 1987. Structure and operation of the feeding apparatus in a colorless eugelnoid, *Entosiphon sulcatum*. J Protozool 34:39–47.

59. Nisbet B. 1974. An ultrastructural study of the feeding apparatus of *Peranema trichophorum*. J Protozool 21:39–48.

60. Triemer RE. 1997. Feeding in *Peranema trichophorum* revisited (Euglenophyta). J Phycol 33:649–654.

61. Triemer RE, Lewandowski CL. 1994. Ultrastructure of the basal apparatus and putative vestigial feeding apparatuses in a quadriflagellate euglenoid (Euglenophyta). J Phycol 30:28–38.

62. Owens KJ, Farmer MA, Triemer RE. 1988. The flagellar apparatus and reservoir/canal cytoskeleton of *Cryptoglena pigra* (Euglenophyceae). J Phycol 24:520–528.