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Primer
Predatory protists

Brian S. Leander

Among the most impactful events in the history of life was the evolutionary origin of phagotrophy over a billion years ago, which triggered the ability of a cell to ingest a particle of organic material, whether dead or alive, as food. Without it, there would be no animals and no plants, let alone the vast number of single-celled lineages of eukaryotes that either photosynthesize and/or consume other organisms to sustain themselves long enough to reproduce. In fact, the most recent common ancestor of all eukaryotes was almost certainly capable of phagotrophy, a trait that fundamentally distinguishes eukaryotes from all other forms of life, namely archaea and bacteria.

The evolution of phagotrophy was predicated by a dynamic proteinaceous cytoskeleton comprising microtubules, actin filaments and associated molecular motors, which together preceded the origin of other distinctive traits of eukaryotes, such as the nucleus, endomembrane system and mitochondria. Phagotrophy also facilitated major evolutionary events that transformed the diversity of life and the planet as a whole, such as multiple origins of photosynthesis and multiple independent origins of parasitism across the tree of eukaryotes.

However, despite these major events, many different lineages of eukaryotes have maintained lifestyles most consistent with their deepest ancestors in the form of free-living predators capable of hunting, killing and consuming other prey organisms. This general mode of nutrition in single-celled eukaryotes has resulted in dynamic predator–prey relationships and a diverse array of traits associated with their feeding apparatus, motility systems and hunting mechanisms. A brief survey of these traits across the tree of eukaryotes is the focus of this primer in order to introduce the reader to some outstanding examples of convergent evolution, structural complexity and behavioral sophistication within the microbial world.

Modes of feeding in predatory protists

The two words used in the main title have definitions of convenience. Use of the term 'protist' here refers to all (mostly single-celled) eukaryotes excluding the following multicellular lineages: green algae/land plants, animals, fungi, brown algae and red algae. Use of the term 'predator' refers to eukaryotes capable of hunting and ingesting relatively large prey cells. Different kinds of predators represent vastly distantly related lineages across the tree of eukaryotes, and this general lifestyle can blend into the definitions of other modes of nutrition, such as stalked suspension feeding and parasitism. Although parasites are smaller than and usually do not kill their hosts, some predators and parasites use the same feeding mechanism to extract nutrients from prey cells and host cells, respectively, which can blur the distinction between the two lifestyles. For instance, the ability to feed like a vampire by piercing the surface of a cell and sucking out its contents as food, known as 'myzocytosis', is found in several different kinds of predators, such as didinid ciliates, colpodellids, colponemids, noctilucoid dinoflagellates and vampyrellid cercozoans, and some marine intestinal parasites, such as archigregarine apicomplexans.

A modification of myzocytosis involves a free-living predator, such as some colpodellids, perforating and entering a prey cell and eating it from the inside out, leaving only an empty shell of what once was. This particular feeding strategy is also found in the zoospores of some parasites, such as perkinsozoan alveolates; after entering the host cell, the zoospores feed on the cell contents and grow a large multicellular sporangium that completely fills the inside of the now exterminated host cell. New zoospores are then released from the mature sporangium and hunt for a new host cell to perpetuate the parasitic lifecycle. Evidence of myzocytosis in the form of perforations in the protective shells of other protists shows up in the fossil record about 750 million years ago.

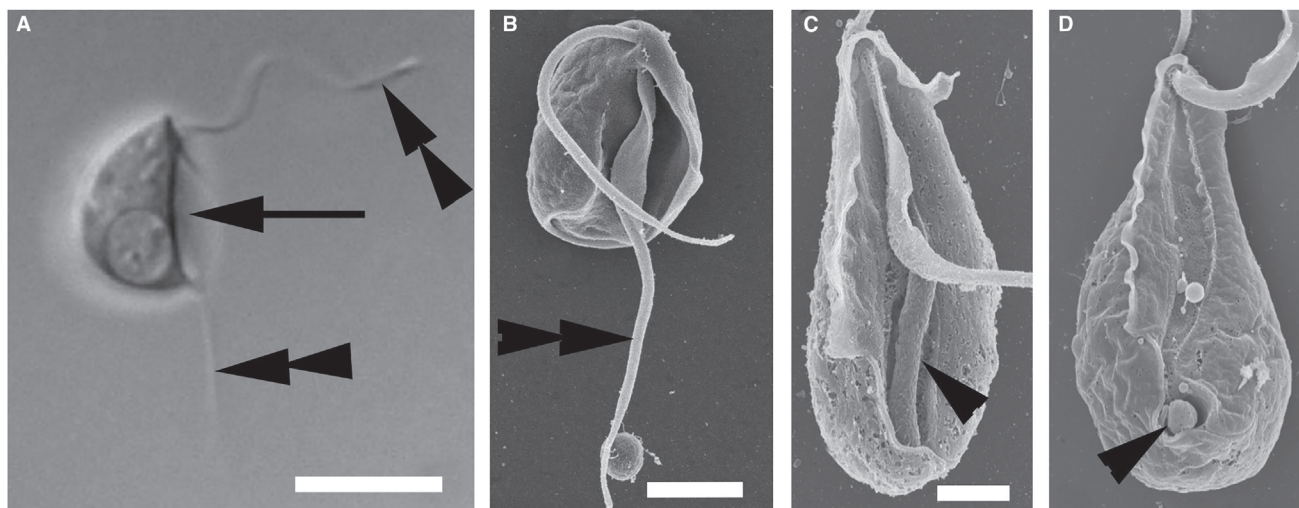Many different lineages of predatory protists acquire food using whole prey cell phagocytosis, which is

Figure 1. Montage of some predatory protists of bacteria and their traits.
(A) Light micrograph of the bicosoecid *Cantina* showing two flagella (double arrowheads) and the ventral groove (arrow) used to feed on bacteria (image courtesy of Yubuki and Leander). (B) Scanning electron micrograph of the excavate *Aduncisulcus* in ventral view showing the ventral groove and two flagella (double arrowhead) (image courtesy of Yubuki and Leander). (C,D) Scanning electron micrographs of the excavate *Kipferlia* showing a rod-shaped bacterium (arrowhead) trapped in the ventral groove (C) and drawn into the mouth-like cell opening (D) (image courtesy of Yubuki and Leander). Scale bars: A = 8 μm, B = 2 μm, C,D = 1 μm.

accomplished in a diverse number of ways. Predators capable of eating large prey cells, such as other eukaryotes, tend to have specialized structures to facilitate this feat. For instance, crawling amoebae ooze dynamic pseudopods around prey cells in their path. Some dinoflagellates unzip specific regions of their otherwise rigid cells to internalize prey cells. Some dinoflagellates (e.g., *Protoperidinium*, *Gyrodinium*) use a large hood-like cell extension, called a 'pallium', to envelop filamentous prey and enzymatically fold it in half several times before ingesting it. Ciliates use a highly expandable oral pocket to ingest prey cells. Many euglenids use a robust system of longitudinal rods and pinwheel-like membranous vanes to grab and pull in prey cells like a Chinese finger trap. Most predatory protists, however, are less than 10 microns long and eat bacteria using a distinctive ventral groove with an opening called a 'cytopharynx' (e.g., jakobids, carpediomonads, percolomonads, malawimonads, bicosoecids, cercomonads and colponemids) (Figure 1); this overall feeding strategy and associated cell morphology spans the tree of eukaryotes and almost certainly represents the traits in the most recent predatory ancestor of the entire group.

As predatory protists evolved more sophisticated feeding strategies, their preferred eukaryotic prey evolved more sophisticated forms of evasion and protection. Multicellular aggregations, for instance, in the form of filaments, prostrate sheets and arborescent arrays create larger body sizes that limit the abilities of protistan predators to feed on them; as such, protistan predators were likely a major selective driver for the independent origins of multicellularity across the tree of eukaryotes. Larger prey sizes then set the stage for a switch from whole-cell phagocytosis to myzocytosis in some predatory protists. Different lineages of prey cells also secrete hard parts as armor, such as thick cell walls, cellulosic thecal plates in dinoflagellates, calcium carbonate coccoliths, chrysophyte scales, siliceous diatom frustules, euglenophyte loricas, ebriid skeletons, foraminiferan tests, agglutinated loricas of tintinnid ciliates, and the shells of euglyphid and arcellinid amoebae. Regardless, some larger predatory protists (e.g., heteronemid euglenids, ciliates and dinoflagellates) can consume armored prey cells whole, move the hard parts through their cells like a pseudo-digestive track, and release the inorganic waste via exocytosis through a specialized anus-like pore, called a 'cytoproct'.

In addition to armor, many prey cells defend themselves with subcellular weapons, called 'extrusive organelles', that come in many different forms, such as the coiled ejectisomes of cryptomonads and the telescopic trichocysts of alveolates. The rapid discharge of extrusive organelles from prey cells serves to both repel a predatory attack like a shield and forcefully propel the prey cell in unpredictable directions and away from the pursuing predator. The effectiveness of this defensive mechanism has even led to peculiar episymbiotic relationships between (verrucomicrobial) bacteria capable of rapidly discharging a tightly coiled thread when disturbed and a large protistan host (e.g., ciliates and euglenozoans); presumably, the network of discharged threads from the episymbiotic bacteria serve to protect the underlying host and the remaining (undischarged) episymbionts from predatory attacks. Of course, predators have evolved weapons of their own to counteract the defenses of their preferred prey, some of which are outlined below.

Subcellular projectiles
Like the defensive extrusive organelles of prey cells, predatory protists use similar subcellular weapons to hunt.
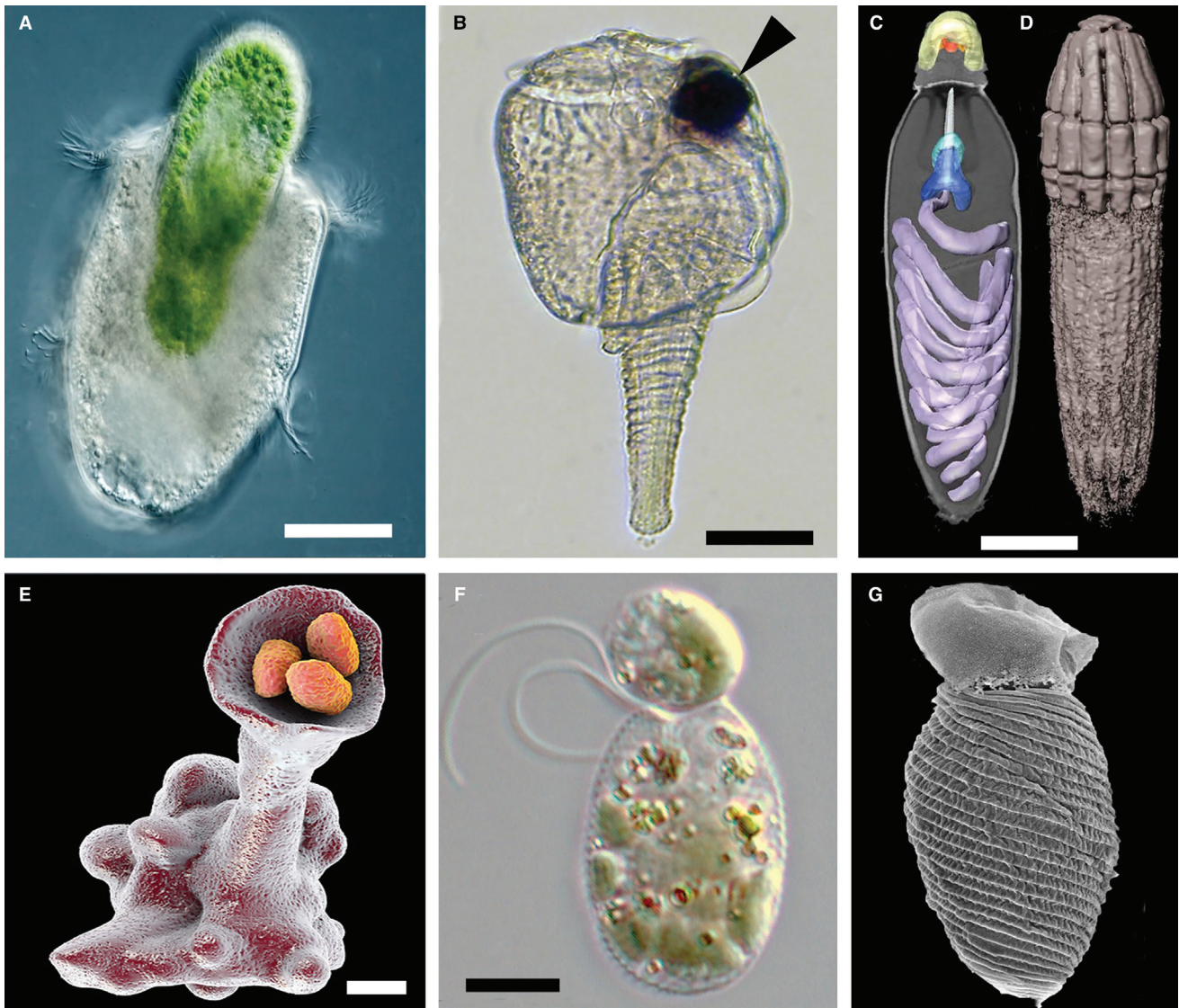
Figure 2. Montage of some predatory protists of other eukaryotes and their traits.
(A) Light micrograph showing the ciliate *Didinium* in the process of catching and consuming the ciliate *Paramecium*; both ciliates use extrusive orga-nelles, namely toxicysts and trichocysts, respectively, to battle each other (image courtesy of Gerald Helbig). (B) Light micrograph of the warnowiid dinoflagellate *Erythropsidinium* showing the eye-like ocelloid (arrowhead); this cell is also equipped with nematocysts used to capture prey cells (image courtesy of Gavelis and Leander). (C,D) Three-dimensional reconstructions of subcellular nematocysts in polykrikoid dinoflagellates (C) and warnowiid dinoflagellates (D); the former are organized as a thread within a pressurized capsule, and the latter contain up to 15 projectiles arranged in a ring (image courtesy of Gavelis and Leander). (E) Scanning electron micrograph showing a predatory ameba using a pseudopod to envelop and consume bacteria (image purchased from Science Photo Library). (F,G) Light micrograph and scanning electron micrograph of the mixotrophic euglenid *Rapaza* (below) in the process of consuming an entire green algal cell (above), namely *Tetraselmis* (image courtesy of Yamaguchi, Yubuki and Leander). Scale bars: A = 5 μm, B = 20 μm, C,D = 2 μm; E = 2 μm, F,G = 5 μm.

A classic example of a predator–prey battle involving the simultaneous discharge of extrusive organelles by both combatants can be observed on a microscope slide when the ciliate *Didinium* attacks the ciliate *Paramecium* (Figure 2A). Once a prey cell has been detected, *Didinium* launches a battery of paralyzing extrusive organelles, called 'toxicysts', from its apical end to immobilize *Paramecium* prior to attempting to slurp it up whole; in response, *Paramecium* discharges a dense network of trichocysts toward *Didinium* as it struggles to escape the attack. This kind of predator–prey interaction is thought to occur between countless numbers of species in marine and freshwater ecosystems around the world, the vast majority of which are either poorly understood or entirely unknown.

The most complex extrusive organelles described so far have been characterized in a specific group of marine predatory dinoflagellates that live amongst plankton, called warnowiids and polykrikoids (Figure 2B). Some of these so-called 'nematocysts' are strikingly similar
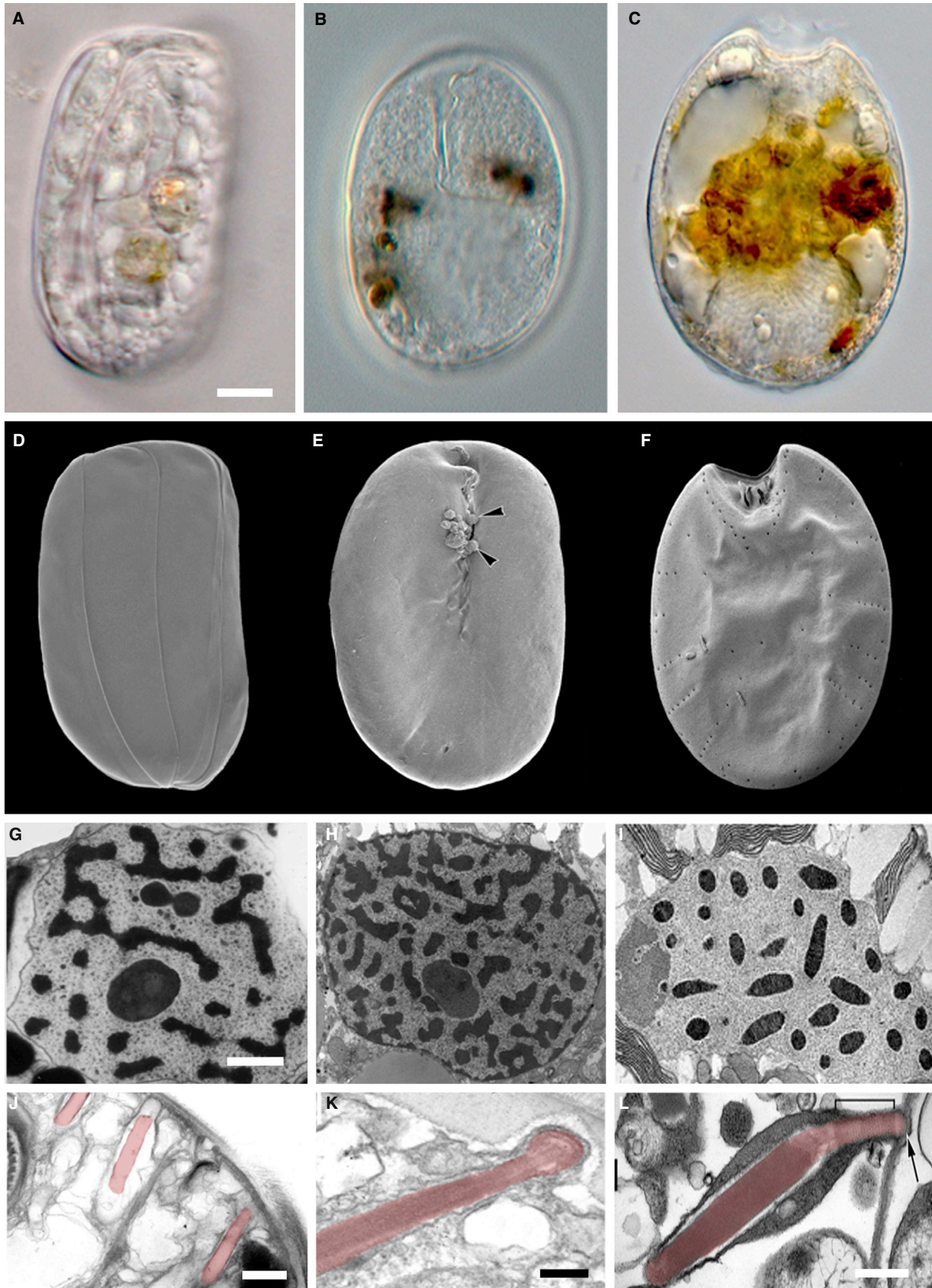
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in form and function to the harpoon-like stinging cells of jellyfish and their closest relatives. These consist of a pressurized subcellular capsule containing a tightly wound thread capable of rapid discharge when disturbed (Figure 2C). A paralytic toxin is delivered when the thread penetrates a prey cell, allowing the predator to feed without the risk of harm by the prey fighting to escape. Although similar in overall form, the nematocysts of dinoflagellates and jellyfish are fundamentally different in molecular composition, development, detailed structure and operation. In jellyfish, the thread is ejected from the capsule, which usually remains within the cell it formed in; in dinoflagellates, both the capsule and the thread are launched toward a prey cell and remain attached to the dinoflagellate cell by a different 'tow line' of unclear origin. These differences reflect convergent evolution over a vast phylogenetic distance; dinoflagellates and jellyfish diverged from each other over one billion years ago.

In addition to nematocysts consisting of a coiled thread within a capsule, some dinoflagellates, namely warnowiids, have even more complex extrusive organelles capable of launching up to 15 projectiles lined up in a circular arrangement similar to a revolver (Figure 2D). These extrusive organelles contain several intricately organized subcomponents with names reminiscent of a machine, such as nozzles, gaskets, shafts, rosettes and stylets; however, their specific functions are essentially unknown. These complex weapons are found in predatory cells with an even more complex subcellular apparatus reminiscent of the camera eyes in cephalopods and vertebrates. This so-called 'ocelloid' consists of a retinal body built from a highly modified chloroplast, a cornea-like layer built from highly modified mitochondria, a crystalline lens presumably built

from starch-like material, and one or more iris-like rings (Figure 2B). We know that warnowiids consume other dinoflagellates as food, so it is tempting to interpret the complexity of the eye-like ocelloid as a light-sensing mechanism for detecting prey cells and aiming the complex revolver-like nematocysts at them. Light-sensing (visual) systems in predatory animals are common (e.g., ocelli and eyes), but they are not common in predatory protists; warnowiid dinoflagellates and possibly some predatory euglenids (e.g., *Urceolus*) are among the only known exceptions. Even though some predatory protists show extraordinary subcellular complexity in their weapons and possibly prey detection, many predatory protists are highly streamlined and have evolved traits that are nearly indistinguishable in very distantly related lineages.

Convergent evolution of predatory protists
The spaces between grains of marine sand are teeming with microbial life, an Earth-enveloping habitat where countless protistan predators are in constant pursuit of prey. This collection of 'meiofaunal' organisms contains very distantly related lineages that represent nearly every major group in the tree of eukaryotes, including tiny multicellular animals that compete with and are about the same size as the single cells of large predatory protists. When examining the overall diversity of predatory protists in this habitat, some species look very similar to one another, mainly because of their streamlined cell shapes when viewed under a light microscope. These particular predators are adapted for moving within the tight spaces between grains of sand by eliminating unnecessary complexity that would get in the way of movement; the cells are essentially flattened ovals capable of gliding along the surfaces of sand grains (Figure 3A–F). Upon closer examination, this type of

protistan predator actually represents several distinct groups that diverged from one another over one billion years ago, such as euglenids (Excavata), dinoflagellates (Alveolata), cryomonads (Rhizaria) and katablepharids (Hacrobia). Euglenids, dinoflagellates and cryomonads each contain a multitude of lineages with extremely diverse morphologies and lifestyles, so the streamlined predatory species that look like flattened ovals under a microscope are not representative of the groups as a whole. Despite their vast phylogenetic distance, these predators look remarkably similar to one another not only in gross cell shape but also at many different subcellular levels. All of these groups have independently acquired a sophisticated feeding apparatus, modes of gliding locomotion, extrusive organelles, robust cell surfaces and chromosomes that remain permanently condensed throughout the cell cycle (Figure 3G–I). Despite these similarities, traits reflecting their 'supergroup' affinities remain. For instance, the dinoflagellate predators have intracellular cellulosic armor, flagellar-based gliding, tubular mitochondrial cristae and lattice-like trichocysts; the euglenid predators have proteinaceous (articulin) pellicle strips, tubular extrusive organelles, flagellar-based gliding and discoidal mitochondrial cristae; the cryomonad predators have an extracellular cell wall, tubular mitochondrial cristae, pseudopod-based gliding and tubular trichocysts (Figure 3G–I)

These groups of predatory protists represent an example of convergent evolution over vast phylogenetic distances; however, the selective forces responsible for their subcellular similarities are poorly understood and currently left only to speculation. Improved knowledge of predators like these will provide evidence necessary for understanding cellular adaptation, subcellular streamlining, subcellular complexity and the overall

Figure 3. Convergent evolution of streamlined predatory protists living in the spaces between grains of marine sand.
Images compare similarities in representatives of three fundamentally different groups of predatory eukaryotes that diverged from one another over one billion years ago: (A,D,G,J) euglenids (Excavata); (B,E,H,K) cryomonad cercozoans (Rhizaria); (C,F,I,L) dinoflagellates (Alveolata). (A–C) Light micrographs showing the flattened oval cells of a benthic predatory euglenid, cryomonad and mixotrophic dinoflagellate, respectively. (D–F) Scanning electron micrographs showing the smooth, robust cell surfaces of a benthic predatory euglenid, cryomonad and dinoflagellate, respectively. Unlike euglenids and dinoflagellates, cryomonads hunt prey cells with pseudopodia (arrowheads) that emerge from a ventral groove. (G–I) Transmission electron micrographs showing the permanently condensed chromosomes in the nucleus of a benthic euglenid, cryomonad and dinoflagellate, respectively. (J–L) Transmission electron micrographs showing the extrusive organelles (pink) in a benthic predatory euglenid, cryomonad and dinoflagellate, respectively. Images from the Leander lab. Scale bars: Images A–F are at the same scale = 5 μm, G–I = 2 μm, J = 1 μm, K,L = 0.5 μm.

evolutionary history within marine interstitial habitats. This research will also bring into the forefront the cellular context needed for interpreting the huge amount of genomic data being generated around the world, especially environmental sequencing surveys of microbial diversity. Nonetheless, continuous interactions between predators like these and their prey create circumstances that have led to major events in the evolution of life, namely transformative shifts in lifestyles and modes of nutrition.

Counterintuitive outcomes of continuous predator–prey interactions

Predatory protists can either have specific prey preferences or indiscriminately eat a wide-range of encountered prey cells. Some predatory protists are limited to eating bacteria because of their small cell sizes under 10 microns and their restricted feeding apparatus, such as the bacteria-sized ventral feeding groove in excavates (Figure 1). Some predatory protists that hunt and consume other eukaryotes can be so finicky that they will eat only a particular strain of a recognized species, such as the euglenid *Rapaza viridis* that, so far as we know, eats only a specific strain of the green alga *Tetraselmis* sp. found in the same tide pool (Figure 2F,G). Predatory prey interactions like these have gone on continuously for thousands if not millions of years. As such, the population of predatory protists is constantly exposed to the properties, including the genetic material, of the population of prey cells they prefer to consume. Over long periods of time, components of the digested prey cells, such as fragments of DNA containing complete genes, can become permanently incorporated into the predatory cells through a process called horizontal gene transfer. This process can ultimately set up the conditions for acquiring organelles, such as chloroplasts, from photosynthetic prey cells leading to a merger of distantly related lineages, with one living inside the other, called 'endosymbiosis'. Endosymbiosis has occurred several times independently across the tree of eukaryotes and has involved incorporated prey

cells that were either cyanobacteria (primary), photosynthetic eukaryotes with cyanobacterial endosymbionts (secondary), or photosynthetic eukaryotes with photosynthetic eukaryotic endosymbionts (tertiary). Ultimately, this process transforms predators into photosynthesizers.

Different stages in the process of endosymbiosis have been described in a wide range of living predatory protists. One of the hallmarks of an intermediate stage in the transformation of a predator to a photosynthesizer is a mode of nutrition called 'mixotrophy', where a protist continues to consume prey cells even though it has already established a photosynthetic symbiont through endosymbiosis. The euglenid *Rapaza viridis*, for instance, is both a predator and a photosynthesizer (Figure 2F,G); however, the robust feeding apparatus found in euglenid predators has become significantly reduced in *Rapaza*, indicating a stage in the gradual loss of the predatory lifestyle. Looking deeper into the diversity of photosynthetic euglenids, which acquired chloroplasts from green algal prey cells, demonstrates a highly reduced, vestigial feeding apparatus and a suite of other subcellular modifications that reflect the transformation from a predatory lifestyle to a photosynthetic lifestyle. For instance, the proteinacious cell surface, or pellicle, in photosynthetic euglenids tends to be protectively thick and rigid when compared to the thin and pliable cell surface of predatory euglenids, which must be able to accommodate the ingestion of large prey cells. This is only one of many examples of how the cell structure of photosynthetic lineages differs significantly from their predatory ancestors. Although counterintuitive, all photosynthetic eukaryotes, including land plants, green algae, red algae and giant kelps, are, from an evolutionary perspective, highly modified predatory protists that were transformed following an endosymbiotic event involving photosynthetic prey cells.

Concluding remarks

The most recent common ancestor of all eukaryotes was almost certainly a tiny predator that used whole-cell phagocytosis to consume bacteria

and archaea as food. This ancestor already had a complex cytoskeleton that supported a ventral feeding groove and two flagella used for gliding along substrates, an overall cell morphology and lifestyle similar to many extant species that span the tree of eukaryotes. As eukaryotes began to diversify and multiply, some lineages expanded their prey preferences and acquired the ability to consume other eukaryotes by either whole-cell phagocytosis or myzocytosis. These predator–prey interactions resulted in selective forces that led to arms races involving subcellular projectiles, like trichocysts and nematocysts, and complex suites of armor, such as shells, scales, cell walls, frustules, loricas and tests. Predatory protists were also selective drivers for the independent origins of multicellularity across the tree of eukaryotes, because prey organisms with larger body sizes are more likely to avoid and survive predatory attacks. Different lineages of predatory protists living in similar environments evolved similar traits, resulting in examples of convergent evolution over vast phylogenetic distances, such as the nematocysts in polykrikoid dinoflagellates and jellyfish and the flattened oval cell shapes in marine sandy habitats. Ultimately, continuous predator–prey interactions set up conditions for multiple endosymbiotic events involving photosynthetic prey cells, which transformed predators into photosynthesizers and created completely new ecosystems across the planet. Because the vast majority of predatory protists are unknown and have only been minimally described with line drawings using light microscopy, there is enormous potential for discoveries using single-cell high-resolution microscopy and comparative genomics/transcriptomics that have implications for our understanding of cell biology, developmental biology, paleontology and evolutionary history.

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Primer
Chytrid fungi

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and Nicolas E. Buchler[2,*]

Fungi have distinguishing traits, such as hyphae and cell walls, that evolved in a fungal ancestor over one billion years ago. Chytrid fungi are some of the earliest diverging fungal lineages that retained features of the opisthokont ancestor of animals and fungi (Figure 1). For example, chytrids make reproductive cells known as zoospores that swim with a motile cilium or crawl like an amoeba. The aim of this primer is to introduce the reader to the life cycle, biology, and ecology of chytrids and other zoosporic fungi. We highlight how chytrids are well positioned to elucidate both the cell biology of the animal–fungal ancestor and the evolution of derived fungal features.

Chytrids exhibit fungal and ancestral features

Life first evolved in the ocean and the last eukaryotic common ancestor (LECA) likely swam and engulfed organic matter via phagocytosis. Based on the shared features found across eukaryotes, LECA had a nucleus, mitochondria, an endo-membrane system, actin and tubulin cytoskeleton, and a centriole for building a mitotic spindle and cilium. LECA gave rise to diverse eukaryotes, some of which remained in aquatic environments and others which colonized land over 500 million years ago. Fungi (e.g. chytrids, rusts, molds, mushrooms, and yeast) are a large eukaryotic kingdom found in many environments and ecological niches. These eukaryotes are decomposers that live on organic matter or as parasites of plants and animals. Fungi are also important symbionts: they are partners of algae and cyanobacteria in lichens or they form mycorrhizae that colonize plant roots and extract water and nutrients from soil in exchange for sugars. The successful expansion and colonization of terrestrial environments by the plant and fungal kingdoms is likely the consequence of a symbiotic relationship between early fungi and photosynthetic algae.

Fungi are closely related to animals through a common opisthokont ancestor that lived in an aquatic environment over one billion years ago (Figure 1). Chytrids and other early-diverging fungi have persisted in this ancestral habitat and have retained traits that make them well adapted to foraging for resources in water. For example, chytrids produce spores (known as zoospores) that lack a cell wall and swim via a motile cilium and/or crawl on surfaces via amoeboid motion (Figure 1). The presence of a centriole and a motile cilium is unique to chytrids and other zoosporic fungi within the fungal kingdom. The cilium is attached to a basal body that contains a classic centriole with nine circularly arranged triplet microtubules that nucleate the axoneme. Similar to many animal cells, chytrids resorb the cilium and the centriole is repurposed as a centrosome to organize the mitotic spindle for nuclear division cycles.

The fungal ancestor evolved new traits ('derived traits') that are shared by all fungi including chytrids. For example, the chytrid life cycle includes a vegetative body ('thallus') with a cell wall and hyphal-like feeding structure known as a rhizoid (Figure 1). Fungal hyphae are branching, filamentous tubes that penetrate organic matter and secrete digestive enzymes to extract nutrients for cell growth. Hyphae grow into substrates by depositing cell wall materials and remodeling enzymes at the hyphal tip via directed vesicle trafficking on a cytoskeletal network. The cell wall is critical because it holds large, hydrostatic pressures caused by internal osmolytes, which generate the biomechanical forces that drive cell wall expansion at the hyphal tip. As in other fungi, the hyphal-like rhizoid is important for colonizing substrates and extracting nutrients to fuel chytrid cell growth.

Chytrid ecology and the evolution of zoosporic fungi

We use the term zoosporic fungi to describe chytrids and other early diverging fungi that have a zoospore stage during their life cycle (Figure 2A). Meta-genomic sequencing has shown that zoosporic fungi comprise much