

Annual Review of Microbiology

Convergent Evolution of Cellular Weaponry

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Annu. Rev. Microbiol. 2026. 80:4.1–4.21

The *Annual Review of Microbiology* is online at micro.annualreviews.org

<https://doi.org/10.1146/annurev-micro-042424-061005>

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Keywords

ballistic organelles, ejectisomes, extrusive organelles, extrusomes, nematocysts, projectiles, trichocysts, toxicysts

Abstract

Cellular weaponry includes a diverse array of ballistic structures, generally referred to as extrusomes, found across the tree of life. Extrusomes have evolved multiple times independently; are morphologically diverse, including structures such as coiled filaments, paracrystalline rods, and telescopic tubules; and facilitate prey capture, defense against predation, and host cell invasion. Their widespread distribution reflects extensive convergent evolution driven by similar ecological pressures. Conversely, homologous extrusomes in closely related taxa can diverge markedly in structure and function following lifestyle shifts, including transitions to parasitism. Alveolates, cryptists, heliozoans, euglenozoans, and numerous other protists possess distinct extrusomes with different discharge mechanisms. Comparable projectiles also occur in multicellular eukaryotes, including cnidarian nematocysts, ctenophore colloblasts, and the infectious spores of parasitic fungi and oomycetes, demonstrating that analogous cellular weaponry has evolved at multiple biological scales. In this article, we synthesize the diversity and phylogenetic distribution of cellular weaponry across eukaryotes, exploring novel examples of convergent evolution at different levels of biological organization.

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1. INTRODUCTION

Cellular weaponry refers to specialized structures built either within a cell or from one or more entire cells that function by rapidly ejecting adhesive threads, toxins, and/or harpoons to capture prey, deter predators, invade host organisms, or interact with the environment (26, 39). These cellular weapons are generally referred to as extrusomes and are common among eukaryotes in the forms of (a) organelles within unicellular organisms (e.g., protists), (b) whole cells of some multicellular organisms (e.g., parasitic fungi and water molds), (c) multicellular structures of some animals (e.g., comb jellies), and even (d) structures in some episymbiotic bacteria (e.g., *Verrucomicrobia*) of unicellular eukaryotes. Most extrusomes serve offensive functions to facilitate prey capture or defensive functions either by directly deterring predators or by allowing the cell to rapidly elude an attacking predator. Some parasitic lineages of eukaryotes, including species of apicomplexans, microsporidia, and oomycetes, have evolved extrusomes to facilitate host cell invasion, thus requiring cellular weaponry to complete the infectious stage of their life cycle. Thread-based extrusomes frequently exhibit ultrastructural and functional similarities in organisms that share similar lifestyle strategies despite vast phylogenetic distances (e.g., divergence occurring over one billion years ago). In fact, comparative analyses of cellular weaponry reveal numerous and remarkable examples of convergent evolution, wherein microbial organisms are shaped by similar environmental pressures (19, 20, 42–44).

Extrusomes are particularly diverse in their development, ultrastructure, mechanisms of discharge, and distribution throughout a cell. Some extrusomes exist as a coiled thread bound within a capsule maintained under high pressure, while others consist of rapidly elongating paracrystalline

rods or telescopic tubules (26). The distribution, abundance, and structural complexity of an extrusome can be used to infer its function. For example, some dinoflagellates (e.g., polykrikoids) possess a relatively small number of complex extrusomes, called nematocysts and taeniocysts, that are used to capture other dinoflagellates as prey (19, 20). In response, the dinoflagellates that are preyed upon utilize extrusomes, called trichocysts, that are numerous, broadly distributed beneath the cell surface, and used in defense against predators; the rapid discharge of a battery of trichocysts either forms a protective barrier or propels the prey cells away from the attacking predator.

The full range of cellular weaponry in eukaryotes, particularly in microbial lineages, encompasses a bewildering display of ultrastructural diversity, independent evolutionary histories, and a convoluted system of specialized terminology. Like in other traits that reflect convergent evolution (e.g., the “wings” of birds, bats, and pterosaurs), many extrusomes in diverse groups of eukaryotes are referred to using similar terms even though they lack homology. Using the same terms for nonhomologous structures (e.g., the “nematocysts” of dinoflagellates and cnidarians; see 20) fosters misinterpretations about evolutionary origins, complicates comparisons of cellular weaponry, and sets up a problematic precedent in the field. This review helps to synthesize current knowledge of cellular weaponry by exploring novel examples of convergent evolution of traits operating at different levels of biological organization. We primarily focus on extrusomes, which function as explosive thread-based projectiles, rather than secretory organelles that slowly release substrates (e.g., mucocysts).

2. THE DIVERSITY AND PHYLOGENETIC DISTRIBUTION OF EXTRUSOMES

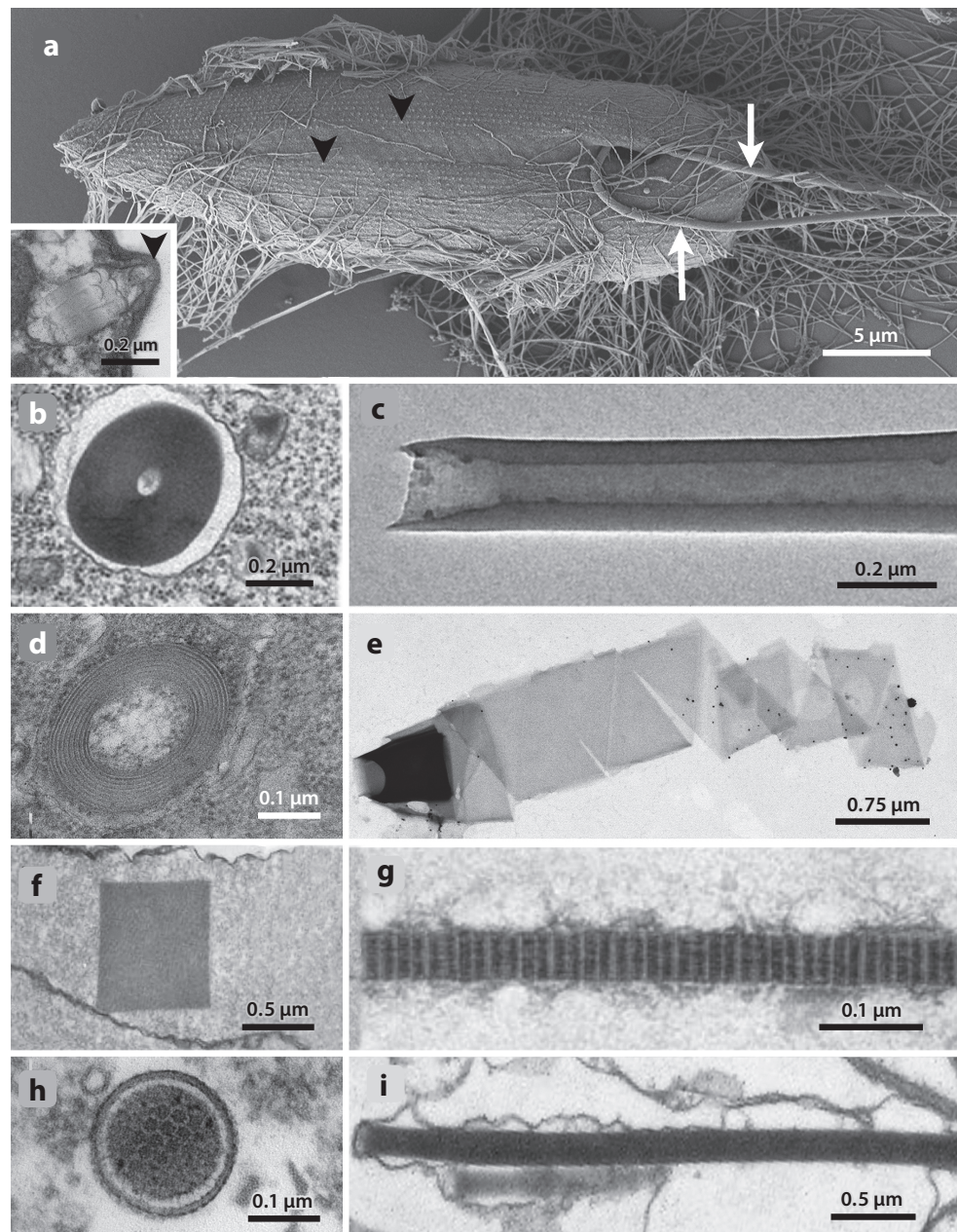
Extrusomes have been categorized on the basis of distinctive morphological traits, with similar structures and functions used to infer the homology of these traits in different lineages of eukaryotes (26, 39). The different forms and modes of discharge in extrusomes include paracrystalline rods that rapidly elongate via a recrystallization process, proteinaceous tubules that discharge telescopically, coiled ribbons that unroll upon discharge, and tightly coiled threads contained within pressurized capsules that fire using either osmotic pressure (e.g., the nematocysts of cnidarians) or contractions of the capsule wall (e.g., the nematocysts of polykrikoid dinoflagellates) (20, 26, 32, 38, 39) (**Figures 1 and 2**). The diversity and complexity of cellular weaponry across the tree of eukaryotes reflect the eukaryotes that possess these weapons, and novel extrusomes are still being discovered as the sampling and characterization of poorly understood lineages of protists continue. Thus, even though certain well-sampled groups of protists, such as ciliates and dinoflagellates, may appear to have more diverse kinds of cellular weaponry, this semblance probably reflects a sampling bias. In fact, extrusomes are so widespread that they have been described to some extent in almost every major group of eukaryotes. Below, we summarize the known diversity of extrusomes.

2.1. Extrusomes of Alveolata: Ciliates, Dinoflagellates, and Apicomplexans

The three major groups of alveolates—namely, ciliates, dinoflagellates, and apicomplexans—have diverse extrusomes that reflect diverse lifestyles and modes of nutrition. Ciliates are mostly predators of bacteria and other protists and are probably the best-represented organisms in ultrastructural and experimental studies of extrusomes. Ciliates utilize several different types of extrusomes for both defensive and offensive functions, sometimes within the same cell. Defensive extrusomes include spindle trichocysts and symbiont-derived epixenosomes, which are extracellular verrucocomicrobial symbionts that rapidly discharge a coiled thread composed of DNA (**Figure 3d,e**); offensive extrusomes include toxicysts, haptocysts, nematocysts, rhabdocysts, and the toxic R-bodies (refractile bodies) within *Caedibacter taeniospiralis*, which is an obligate bacterial

endosymbiont of *Paramecium* (10, 26, 51, 53, 62, 63, 68, 75, 80, 106) (**Figure 1d,e**). Extrusomes in ciliates have been extensively studied for over a century, with comprehensive reviews provided by Hausmann (26), Rosati & Modeo (75), and Plattner (63).

Apicomplexans form a diverse group of obligate animal parasites that thrive in marine, fresh-water, and terrestrial ecosystems and that include significant pathogens of humans, livestock, and shellfish. These parasites, which use secretory vesicles, called rhoptries, that are discharged using



(Caption for **Figure 1** appears on following page)

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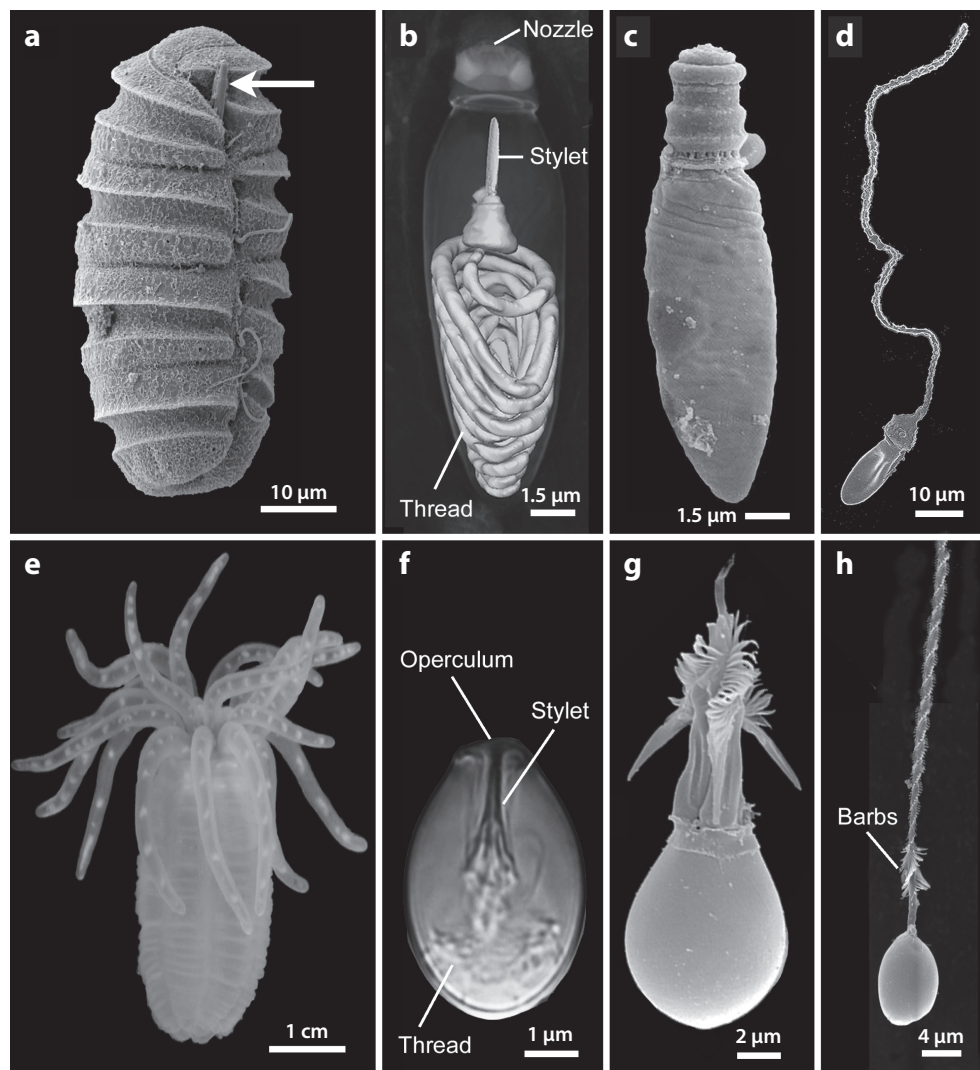
Examples of defensive extrusomes in the form of distinctive organelles found within different lineages of microbial eukaryotes. (a) A scanning electron micrograph of a katablepharid cell (*Platychilomonas* sp.) shows two flagella (arrows) and a network of discharged ejectisomes (i.e., the fine threads) used in defense. The docking sites (arrowheads) for the ejectisomes are positioned below the cell surface (i.e., in the periplast) and arranged as small bumps (0.2 μ m) in longitudinal rows. (Inset) A transmission electron micrograph (TEM) of an undischarged ejectisome, showing a folded thread within a capsule below a docking site (arrowhead) in the periplast. (b) A TEM depicts a cross section through a relatively large undischarged ejectisome near the gullet of a cryptomonad. (c) A negative-stain TEM displays a tubular ribbon structure of a fired ejectisome in cryptomonads. (d) In this TEM, a cross section through an undischarged R-body (refractile body) within the ciliate *Paramecium* shows the concentric layers of a coiled ribbon. (e) A negative-stain TEM features the coiled ribbonlike structure of a fired R-body. (f) A TEM of a cross section through an undischarged trichocyst in a dinoflagellate (*Peridiniella*) shows the square shape and homogeneous composition. (g) In this TEM, a longitudinal section through a fired trichocyst in a dinoflagellate (*Peridiniella*) depicts the striated latticelike crystalline structure that results from the expansion and elongation of the extrusome. (h) A TEM of a cross section through an undischarged tubular extrusome in a euglenozoan (*Olkasia*) shows the electron-dense ring around a cruciform core. (i) A TEM displays a longitudinal section through an undischarged tubular extrusome in a euglenozoan (*Bibospites*). Panels b and c adapted with permission from Rhiel & Westermann (71). Panels d and e adapted with permission from Schrällhammer (79).

specialized cellular machinery, called the rhoptry secretory apparatus, for host cell attachment and invasion, have been particularly well-characterized in medically relevant genera such as *Plasmodium*, *Toxoplasma*, and *Cryptosporidium* (12, 83). Although the rhoptries of apicomplexans secrete proteins that modify the host cell and that are, therefore, not rapidly discharged, thread-based extrusomes, the biogenesis and transport pathways of rhoptries are genetically homologous with those of the explosive trichocysts of ciliates, despite their structural and functional dissimilarities (83). Both apicomplexans and ciliates contain a rosette of intramembranous particles that facilitate the discharge of secretory vesicles at the cell surface. The formation of the rosette in both lineages is mediated by a set of homologous proteins; however, how the rosette interacts with the secretory vesicles in ciliates and apicomplexans differs. In ciliates, this rosette interacts directly with the explosive trichocyst, while in some apicomplexans, the discharge of the secretory material contained within rhoptries is mediated by an intermediary structure called the apical vesicle that contacts the rosette at the cell surface and that forms part of the rhoptry secretory apparatus together with the rosette.

Dinoflagellates also display a diverse array of extrusomes that reflect diverse lifestyles, including predatory, mixotrophic, and phototrophic modes of nutrition (70). Photosynthetic dinoflagellates have mucocysts that release mucoid material and trichocysts that are used for defense, such as the trichocysts of ciliates. Trichocysts (Figure 1f,g) are ubiquitous among dinoflagellates, including heterotrophic lineages, often numbering in the hundreds within a single cell, and are relatively simple in structure compared with the more structurally complex weaponry observed in some predatory dinoflagellates (19, 20, 70). Predatory dinoflagellates exhibit remarkable cellular complexity associated with structures specifically adapted for the efficient capture of prey (19, 20, 70). Warnowiid and polykrikoid dinoflagellates have evolved intricate ballistic systems composed of one or more types of extrusomes, including nematocysts and taeniocysts, which aid in prey capture (19, 20, 30–32, 46) (Figure 2a–d). In polykrikoids, for example, each nematocyst consists of a coiled thread connected to a stylet and housed within a capsule (20) (Figure 2b). Maintained under high pressure, the stylet pierces the capsule in response to a stimulus, rapidly freeing the coiled thread, which can then pierce a prey cell. In polykrikoids, a specialized nematocyst–taeniocyst complex facilitates this process: The taeniocyst discharges first and adheres to the prey cell, and then the discharge of the nematocyst punctures the prey to immobilize or kill it (19, 20) (Figure 2a–d).

Moreover, *Nematodinium* (Warnowiaceae) has “nematocysts” that are likely homologous to those in polykrikoids, but they consist of several (11–15) elongated projectiles arranged in a circle, resembling a Gatling gun, and lack both the coiled tubule and the taeniocyst (20). Other lineages of dinoflagellates also exhibit novel extrusomes, such as the “rhabdosomes” of dinophysoids and the “vlimatocysts” of *Oxyrrhis marina* (70, 91). These extrusomes do not resemble those previously described in other eukaryotes, suggesting they represent novel subcellular weaponry independently acquired within these lineages. However, their specific functions, development, and discharge mechanisms remain unclear. For instance, the rhabdosomes of dinophysoids have never been observed in their discharged state, rendering it speculative whether they truly act as projectile organelles.

Dinoflagellate trichocysts (**Figure 1f,g**) consist of extending paracrystalline rods that discharge in defense against predators, much like the spindle trichocysts of ciliates (70). Dinoflagellate



(Caption for **Figure 2** appears on following page)

Figure 2 (Figure appears on preceding page)

Convergent evolution of nematocysts, which are primarily offensive extrusomes (i.e., used for capturing prey) in the form of a pressurized capsule containing a stylet and a tightly wound thread capable of explosive discharge. Despite their independent origins, the nematocysts of polykrikoid dinoflagellates (*a–d*) and cnidarians (*e–h*) are morphologically and functionally similar. (*a*) A scanning electron micrograph (SEM) of *Polykrikos* shows an undischarged taeniocyst (arrow) docked near the apical end of the cell. Taeniocysts work in tandem with nematocysts to capture prey cells using the spear-like stylet and a towline (i.e., the everted thread); the nematocyst is positioned beneath a taeniocyst in the same linear orientation and fires after the initial explosion of the taeniocyst. (*b*) A 3D reconstruction of a polykrikoid nematocyst uses focused ion beam SEM and depicts the pressurized capsule, nozzle, stylet, and inverted thread. (*c*) An SEM of an undischarged polykrikoid nematocyst displays the surface details of the capsule and nozzle. (*d*) An SEM of a fired polykrikoid nematocyst shows the surface details of the capsule and thread. (*e*) A light micrograph of the anemone *Nematostella vectensis* displays an array of tentacles armed with cnidarian nematocysts. Nematocysts use the spear-like stylet and a towline (i.e., the everted thread) to capture prey cells; the nematocyst is positioned beneath a taeniocyst in the same linear orientation and fires after the initial explosion of the taeniocyst. (*f*) A differential interference contrast micrograph of an undischarged nematocyst in *Hydra* shows the pressurized capsule, operculum, stylet, and inverted thread. (*g*) An SEM of a discharged cnidarian nematocyst depicts the surface details of the capsule and barbs. (*h*) An SEM of a fired nematocyst in the cnidarian *Catostylus mosaicus* shows the surface details of the capsule, barbs, and thread. Panels *a–d* adapted with permission from Gavelis et al. (20). Light micrograph in panel *e* provided by Robert Aguilar, Smithsonian Environmental Research Center. Panel *f* adapted with permission from Wang et al. (95). Panel *g* adapted with permission from Thomas Holstein. SEM in panel *h* provided by Meredith Peach and Kylie Pitt.

trichocysts are similar in structure, organization, and function to the trichocysts of ciliates, suggesting they are homologous with one another. Moreover, colponemids, which form the closest sister lineage to alveolates, also use toxicysts and trichocysts to capture prey (35, 88). These data, alongside evidence that homologous pathways facilitate the development of the ciliate trichocysts and apicomplexan rhoptries, support the inference that trichocysts are homologous across alveolates (83).

2.2. Extrusomes of Discoba: Euglenozoans and Heteroloboseans

The Euglenozoa are, in part, characterized by the widespread presence in many taxa of cruciate tubular extrusomes (**Figure 1b,i**), sometimes referred to as “trichocysts” even though they lack notable structural similarity with the trichocysts of alveolates (45, 82). They have predominantly been studied in euglenids; however, these studies are not as in-depth as those of extrusomes in different lineages of eukaryotes, such as alveolates and cryptists. These extrusomes are often present in highly organized batteries in diplomonids, kinetoplastids, euglenids (e.g., *Entosiphon*, *Teloplecta*), and epibiont-possessing symbiontids (7, 8, 66, 82, 87, 94, 102, 104), but these extrusomes can also be dispersed throughout the cell in some euglenozoans (e.g., *Olkasia polycarbonata*, *Neometanema parovale*) (49, 60). The function of these extrusomes is unknown, but a role in either prey capture or evading predators, as other extrusomes play, is inferred (52). Photosynthetic euglenids lack tubular extrusomes and instead possess mucocysts that are arranged in rows and that secrete mucilaginous material through pores situated between pellicle strips (45). In contrast, petalomonads, the earliest branching lineage of euglenids, have not been observed to have tubular extrusomes and instead have large, globular extrusomes that do not appear to be homologous with tubular extrusomes and that may represent a third type of extrusome within euglenids alone (45, 48).

Several flagellated members of the Heterolobosea have extrusomes within their cytoplasm, though these differ in structure from the tubular extrusomes of euglenozoans (e.g., *Stephanopogon minuta*, *Multisulcus malaysiensis*) (65, 82, 103). Thus far, no studies have attempted to resolve the evolutionary origins and patterns of development for the extrusomes of either heteroloboseans or euglenozoans despite the close phylogenetic relationship of these two groups within the Discoba.

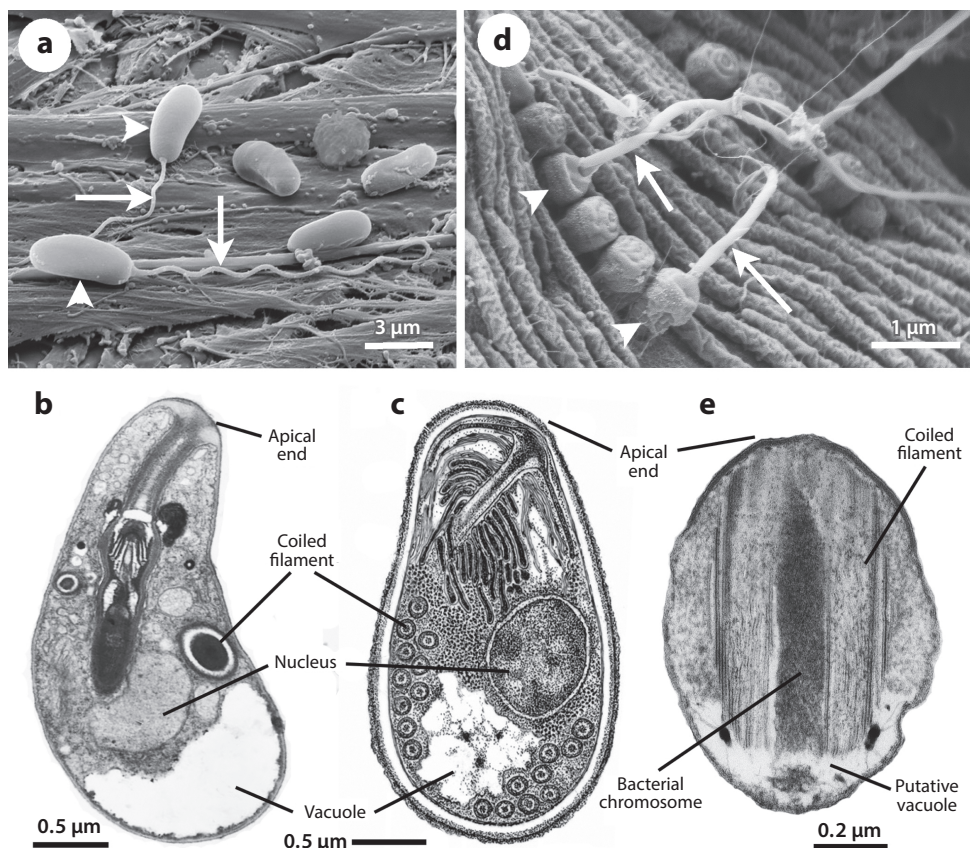


Figure 3

Convergent evolution of single pressurized cells (in contrast to organelles) containing a tightly wound filament that is capable of explosive discharge and that functions either to facilitate host infection in some eukaryotic parasites or to defend a eukaryotic host cell and its episymbiotic bacteria. The three examples shown come from lineages that diverged over a billion years ago. (a, c) Microsporidian spores (parasitic fungi) contain a pressurized capsule (arrowheads) and a coiled filament (i.e., the polar tube, indicated by arrows) used to inject the parasite's nucleus into a host cell (e.g., an arthropod cell). (b) *Haptoglossa* (a parasitic oomycete) uses gun cells to infect host cells (e.g., nematode cells). (d, e) Episymbiotic verrucomicrobial bacteria or epixenosomes (arrowheads) on the euglenozoan *Bibiospites* rapidly discharge a tightly wound filament (arrows), which contains its chromosome, through an apical pore. Scanning electron micrograph in panel a adapted with permission from Eye of Science/Science Photo Library. Panel b adapted with permission from Casal et al. (11). Panel c adapted with permission from Glockling & Beakes (21). Panel e adapted from Breglia et al. (7) (CC BY 2.0).

Although ultrastructural studies of jakobids remain few, these protists have not been observed to contain extrusomes (84).

2.3. Ejectisomes of Cryptomonads, Katablepharids, and Prasinophyte Green Algae

Cryptomonads and katablepharids (cryptists) utilize defensive cellular weaponry known as ejectisomes to rapidly escape from predators (26, 38, 39, 47, 56, 69, 72) (Figure 1a–c). Similar defensive weapons, also referred to as ejectisomes, are present in the prasinophyte green algae

(Archaeplastida) and represent a remarkable example of convergent evolution, as they exhibit striking similarities in ultrastructure and cellular organization with the ejectisomes of cryptists (98). In fact, cryptomonads, katablepharids, and prasinophyte green algae possess two types of ejectisomes that are similar in structure but differ in size and organization: (a) a discrete quantity of large ejectisomes organized near the anterior region of the cell or within a ventral gullet and (b) numerous small ejectisomes distributed around the periphery of the cell (39, figure 8) (**Figure 1a,b**). These ejectisomes were once inferred to be homologous in all three lineages, not only because they are similar in structure, function, and distribution within the cell but also because archaeplastids (e.g., prasinophyte green algae) and cryptists (e.g., cryptomonads and katablepharids) are sister groups in the tree of eukaryotes (39, 89, 107). However, the ejectisomes of prasinophyte green algae differ markedly in molecular composition, consisting of core histones and *N*-acetylglucosamine polymers, and are restricted to a single genus (*Pyramimonas*) (2). These findings provide evidence that the ejectisomes of prasinophyte green algae are not homologous to the ejectisomes of cryptists (i.e., cryptomonads and katablepharids) (98).

Moreover, these ejectisomes, particularly those in cryptomonads, share structural similarities with the extrusive R-bodies produced by the bacterial symbionts of the ciliate *Paramecium*, suggesting that ejectisomes are derived from R-bodies in symbiotic bacteria (3, 26, 100) (**Figure 1d,e**). As a result, the ejectisomes of cryptomonads, katablepharids, and prasinophyte green algae have become the focus of numerous experimental and molecular studies aimed at clarifying their evolutionary relationships with R-bodies (2, 3, 71, 72, 100). Cryptomonads and katablepharids possess the ejectisome-associated *Tri* gene family, members of which are homologous with genes involved in *Paramecium* R-body formation; however, these genes were likely acquired via horizontal gene transfer from bacteria capable of producing R-bodies, and the ejectisomes themselves do not appear to be derived from symbiotic bacteria, as multiple ultrastructural studies indicate that they develop in the Golgi apparatus and are therefore autogenous cytoplasmic organelles (3, 100).

2.4. Convergent Evolution of Extrusomes in Heliozoans (Actinophryid and Centrohelid Amoeboae) and Cercomonads

Actinophryids and centrohelids provide a striking example of morphological convergence leading to the independent evolution of extrusomes with similar functions (27, 39). Considered heliozoans when defined solely by morphological traits rather than by molecular phylogenetic position, centrohelids (Haptophyta) and actinophryids (Stramenopiles) are two distantly related groups of sunlike amoebae. Both groups have radial symmetry with numerous spinelike pseudopodia, known as axopodia, which are supported by distinct bundles of microtubules and radiate outward from the central cell body (26, 27, 36). Both lineages use their axopodia to capture and draw prey cells into their cytoplasm by phagocytosis. Despite their vast evolutionary distance (i.e., they diverged over 1 billion years ago), centrohelid and actinophryid heliozoans share similar body plans and have developed similar extrusomes embedded beneath the plasma membrane of their axopodia.

These extrusomes have evolved independently within each lineage and are referred to by different terms: dense granules in actinophryids versus kinetocysts in centrohelids. Both of these extrusomes rapidly discharge and adhere to prey cells (78). Unlike actinophryids, which seem to rely solely on the adhesive function of their extrusomes to capture prey, centrohelids use kinetocysts to excrete a toxic substance that helps immobilize prey cells. Observations of the centrohelid *Raphidiophrys contractilis* demonstrate that the cilia of prey *Paramecium* stop beating and do not discharge trichocysts in defense when in contact with the axopodia of *R. contractilis*, suggesting a similar toxic effect to that induced by ciliate toxicysts (78). Despite differences in the details of how these heliozoan extrusomes attack prey cells, the convergent evolution of extrusomes in these two lineages allows slow-moving amoeboid predators to capture dynamic prey cells.

A superficially similar system of extrusomes has been observed in the cercozoan *Aurigamonas solis*, which is a radially symmetrical amoeboid flagellate that uses special pseudopods, called haptopods, to capture prey (92). Each haptopod is supported by microfilaments instead of microtubules and contains an offensive extrusome called a haptosome, which is inferred to be involved in prey capture. *A. solis* is not the only cercozoan to have extrusomes that resemble those of other protists, despite being independently derived. In fact, organelles referred to as kinetocysts, microtoxicysts, trichocysts, and osmiophilic bodies, among others, have been observed in select cercozoan taxa, but findings are limited to only a few detailed ultrastructural studies (29, 37, 54, 55). Therefore, the mechanisms of discharge, developmental pathways, evolutionary relationships, and functions of these extrusomes remain unknown.

2.5. Ballistic Structures in Animals, Parasitic Fungi, and Oomycetes

Cnidarians and ctenophores are distinct lineages of gelatinous animals that have independently evolved specialized projectile systems within their tentacles to capture prey and defend against predators (81). Cnidarians use specialized cells called cnidocytes containing diverse kinds of sub-cellular cnidae (e.g., nematocysts and spirocysts) (**Figure 2e–h**). Although undischarged cnidae consist of a pressurized capsule containing an inverted thread, they differ in origin, development, size, and ultrastructure to the nematocysts in dinoflagellates (19, 20, 23, 26, 58, 59, 61, 96). When cnidian (penetrant) nematocysts are triggered by a mechanical, electrical, or chemical stimulus, a rapid increase in osmotic pressure within the capsule everts the thread so fast that it punctures prey cells, delivering a toxic load that either immobilizes or kills the prey and protects the fragile predator from injury. While these nematocysts are only produced by cnidarians, some distantly related animals (e.g., aeolid nudibranchs, flatworms, and ctenophores) can steal and maintain nematocysts within their own tissues as stolen weapons (i.e., kleptocnidae) from their cnidian prey (19, 23). In contrast to cnidarians, ctenophores use novel multicellular structures, called colloblasts, that are localized to their tentacles and that use a spring-loaded mechanism to release adhesive material onto prey cells (50, 93). Despite their shared function as thread-based projectiles that facilitate prey capture in gelatinous animals with tentacles, ctenophore colloblasts and cnidian nematocysts are built at different levels of biological organization (i.e., entire cells in the former case and organelles in the latter) and use different mechanisms of action.

Some parasitic oomycetes and fungi—namely, *Haptoglossa* and microsporidia—are intracellular parasites that have independently evolved a similar ballistic mechanism involving the restructuring of a single cell to facilitate host cell invasion (19, 39). Both *Haptoglossa* and microsporidia use a rapidly everted injection tube from a pressurized spore capsule to infect a new host (**Figure 3a–c**). *Haptoglossa* parasitizes nematodes via specialized gun cells that rely on changes in osmotic pressure to puncture and invade host tissue, transferring the nuclei of the parasite into host cells; microsporidia use a polar tube maintained under high pressures to serve the same function. The origin, structure, and function of polar tubes in microsporidia were thoroughly reviewed by Chen et al. (13). The structure and function of gun cells in *Haptoglossa* were reviewed by Kugrens et al. (39) and Beakes et al. (5).

2.6. Extrusomes in Other Eukaryotes

Many other lineages of eukaryotes utilize some form of cellular weaponry, including the relatively undersampled provirids, hemimastigophorans, ancyromonads, apusomonads, telonemids, and malawimonads (6, 14, 22, 28, 41, 57, 90, 97, 99). Some of these extrusomes reflect novel organization and mechanisms of action, such as the spherical discobolocysts of chrysophyceean algae (e.g., *Chromulina georgesiana*, *Cyclonexis annularis*, *Ochromonas* spp.), which are situated

between plastids and the cell membrane (24, 26). Provorids and some hemimastigophorans (e.g., *Hemimastix amphikineta*), for example, possess novel, structurally similar extrusomes, implying a shared evolutionary origin (6). These organelles have an electron-dense base that tapers into needlelike tips and are situated within feeding structures in their respective lineages (6, 41). In provorids, the extrusomes (i.e., ampulosomes) are positioned within the ventral groove, may be adhesive, and help immobilize prey. Other hemimastigophorans (e.g., *Meteora sporadica*, *Solarion arianae*) contain kinetocyst-like globular extrusomes, called celestiosomes, that facilitate feeding (14, 90). In fact, almost every major group of eukaryotes contains at least some representatives that use a putative extrusome for survival, with the exception of breviate, amoebozoans, CRuMs [i.e., an acronym for Collodictyonidae (synonym, Diphyllidae), Rigifilida, and *Mantamonas*], and metamonads. Whether these lineages actually lack extrusomes or they have simply not yet been observed in these organisms is unclear. Nonetheless, the appearance that extrusomes are significantly more common in flagellated or ciliated organisms than in amoeboid organisms is interesting.

3. FUNCTIONS OF EXTRUSOMES

The function of cellular weaponry in eukaryotes often reflects the lifestyle of the organism and generally falls under one of three categories: predation, host invasion, or defense from predators. Determining the exact function that an extrusive organelle, cell, or multicellular structure serves can be challenging without detailed experimental studies or direct observations of behavior. Because many lineages, particularly those of uncultivated microbial eukaryotes, are understudied, the function and development of extrusomes in these taxa are not understood. However, the abundance and localization of extrusomes within an organism can provide some insight into their function. Extrusomes used for prey capture are often fewer in number and localized to specific positions in the organism (e.g., the tentacles of cnidarians, ctenophores, and suctorian ciliates) (**Figure 2a**), whereas extrusomes used in defense are more numerous and broadly distributed around the periphery of the organism (e.g., the trichocysts of dinoflagellates, the ejectisomes of katablepharids, and the kleptocnidae in nudibranchs) (23) (**Figure 1a**).

3.1. Prey Capture

Extrusomes that facilitate prey capture have evolved independently multiple times across the tree of eukaryotes. Many predators use extrusomes to either entangle or harpoon prey cells, preventing their escape by pulling them into the feeding apparatus for phagocytosis, as is observed with the extrusomes of heliozoan amoebae, the taeniocysts of dinoflagellates, and the haptocysts of suctorian ciliates (26, 27). Other predators use extrusomes that contain toxic substances to immobilize or kill their prey, such as the toxicysts of haptorian ciliates (9, 51, 75). Extrusomes in predatory protists typically target large, motile eukaryotic prey cells, such as ciliates, dinoflagellates, and euglenids, and are rarely observed as a strategy to capture bacterial prey (14). This correlation reflects a reduced evolutionary pressure to develop complex cellular weaponry to hunt slow-moving bacterivorous cells. Nonetheless, the celestiosomes of the hemimastigophorans *M. sporadica* and *S. arianae*, located within long arms supported by microtubules, similar to those in heliozoans, have been inferred to facilitate the capture of bacterial prey cells (14, 90).

3.2. Host Invasion

The release of proteins and lipids via saclike extrusive organelles (e.g., apicomplexan rhoptries) is a common strategy used by parasitic eukaryotes to invade their hosts, either by facilitating adhesion to host tissues or by protecting parasitic eukaryotes against the host's immune response (e.g., by forming a parasitophorous vacuole) (12, 83, 101). However, some parasitic protists have

independently evolved subcellular projectiles to facilitate host invasion. As mentioned above, *Haptoglossa* and microsporidia rapidly discharge injection tubes from a pressurized spore to invade their respective hosts (e.g., nematodes) and complete their parasitic life cycle (39). Similarly, myxozoans, which are highly reduced parasitic cnidarians of fish, use modified nematocysts for host attachment (1, 25, 96), and *Helicosporidium*, which is a parasitic lineage of green algae, discharges a specialized filament to attach to the cell surface of its insect host (67, 86).

Other kinds of extrusomes that facilitate parasitic lifestyles are homologous to those in their free-living relatives, such as in certain parasitic ciliates and potentially in the parasitic dinoflagellate *Hematodinium* (16, 18, 101). *Ichthyophthirius multifiliis* and *Philasterides dicentrarchi*, for example, are ciliates that use toxicysts and trichocysts, respectively, to parasitize fish (16, 101). Molecular and morphological evidence suggests that the toxicysts in *I. multifiliis* result in the necrosis of host tissue during the parasitic stage of the life cycle and release proteins (e.g., leishmanolysins) that counteract host immune defenses (101). *P. dicentrarchi*, a ciliate that has independently evolved a parasitic lifestyle on fish, uses both trichocysts and mucocysts to release *O*-glycosylated matrix proteins that facilitate evasion of the host immune response (16).

3.3. Defense Against Predators

Extrusomes that function in defense are common in protists and often distributed in large numbers throughout an individual cell (**Figure 1a**); most of them develop endogenously into elongated rods or tubules that dock beneath the plasma membrane, such as the trichocysts of free-living alveolates, the extrusomes of euglenozoans, and the ejectisomes of cryptists (26, 39) (**Figure 1**). The presence of defensive extrusomes in protists reflects a “microbial arms race” with the cellular weaponry of predatory protists, such as when prey ciliates (e.g., *Paramecium*) deploy batteries of trichocysts in response to the harpoon-like toxicyst fired by predatory ciliates (e.g., *Didinium*) (20, 75, 85).

Defensive extrusomes can also exist as independently acquired bacterial episymbionts of protistan hosts, such as the verrucomicrobial episymbiotic bacteria that occur on some species of the ciliate *Euplotidium* and on the symbiontid euglenozoan *Bibospites bacati* (7, 45, 53, 73–77, 104) (**Figure 3d,e**). The verrucomicrobial episymbionts develop into distinct spherical bodies, called epixenosomes, that are arranged in rows along the longitudinal axis of the host and that consist of a tightly wound thread around a central core of DNA (**Figure 3d,e**). The presence of epixenosomes is critical for *Euplotidium itoi* to defend itself against the toxicysts of haptorian ciliates (76). Cells of *E. itoi* equipped with epixenosomes are immobilized but not ingested when exposed to the predatory ciliate *Litonotus lamella*; however, cells of *E. itoi* without epixenosomes are readily consumed by *L. lamella* under identical culture conditions. Although the mechanism of action by which epixenosomes defend their hosts against predation is unknown, they may hamper the ability of predatory ciliates to detect their prey after immobilization.

4. CELLULAR WEAPONRY OPERATING AT DIFFERENT LEVELS OF BIOLOGICAL ORGANIZATION

Cellular weapons can operate at different biological levels of organization even though they share key structural and functional features across eukaryotes (19). Extrusomes involving the eversion of a coiled thread under osmotic pressure within a capsule can be built from organelles (e.g., the nematocysts of polykrikoid dinoflagellates and cnidarians, shown in **Figure 2**), bacterial episymbiotic cells (e.g., the epixenosomes of *Bibospites* and some *Euplotidium* spp., shown in **Figure 3d,e**), and entire eukaryotic cells (e.g., microsporidian spores and the gun cells of *Haptoglossa*, shown in **Figure 3a–c**) (19, 39). Multicellular organisms can possess weaponry built from multiple cells, such

as the colloblasts of ctenophores. Colloblasts are composed of a spring-loaded collocyte, which functions as the main body of the projectile, and one to several cap cells, which contain adhesive granules (50, 93). Upon contact with prey organisms, a colloblast will rapidly discharge, releasing adhesive granules from the cap cells, which effectively glue the prey organism to the surface of the tentacle (50). Unlike cnidarian nematocysts that use toxins to capture prey, colloblasts of ctenophores accomplish this function solely through adhesion mechanisms.

5. MECHANISMS OF DISCHARGE

The stimuli and mechanisms associated with the rapid discharge of extrusomes are diverse (26). The firing of extrusomes can be triggered by changes in environmental factors, such as light, temperature, and electrical and chemical gradients, as well as by direct contact with physical objects. Sensitivity to these stimuli corresponds to the functional role of the extrusome. For example, defensive projectiles, such as ejectosomes in cryptists, and offensive projectiles, such as toxicysts in ciliates, are discharged in response to the presence of other organisms. The mechanisms of extrusome discharge remain poorly understood in most organisms that possess these structures, even when the ultrastructure of the extrusome is relatively well-characterized. The diversity of discharge mechanisms reflects the convergent evolution of these structures over vast phylogenetic distances among eukaryotes. Consequently, homologous extrusomes can exhibit divergent discharge mechanisms, while structurally similar extrusomes may share similar discharge processes despite their independent evolutionary origins.

For instance, nematocyst-like extrusomes, microsporidian spores, and the gun cells of *Haploglossa* operate by generating pressure inside the capsule to rapidly evert a coiled tubule (Figures 2 and 3). These extrusomes require the generation of considerable force during discharge because they must ultimately puncture their target organism to either deliver toxins or facilitate parasite propagation. The mechanisms by which pressure is generated inside these capsules vary, reflecting independent evolutionary pathways. Cnidarians, for instance, generate pressure within their nematocysts using an osmotic propellant that binds cations and creates high internal osmotic pressure (1, 59), while microsporidia utilize the swelling of intracellular vesicles to drive extrusion of the polar tubule (15, 39). The mechanism generating pressure in the nematocysts of polykrikoid dinoflagellates is not fully understood, but it may involve the contraction of stratified fibrous material within the capsule walls (20). Other extrusomes, such as the rhabdocysts of some ciliates, also rely on a buildup of pressure through vesicle swelling to facilitate the extrusion of a thread (26, 68).

The colloblasts of ctenophores differ from other extrusomes in that they do not rely on the exocytosis of material to discharge and adhere to prey. Instead, the secretory granules present in the cap cell of the colloblast rupture to release adhesive when in contact with prey (17). The exact mechanism by which colloblasts discharge is still largely unknown; whether the spring-loaded collocyte of each colloblast fires in response to a physical stimulus or releases once the colloblast is in contact with prey, acting as a tether, is unclear (93).

Although the precise mechanisms underlying the discharge of most types of extrusomes remain unknown beyond what can be inferred from their ultrastructure, the firing of extrusomes in heliozoans, the discharge of trichocysts and toxicysts in alveolates, and the eversion of polar tubules in microsporidia are all modulated by an influx of extracellular calcium ions into the cell in response to stimuli (4, 34, 36, 39, 63, 64). Calcium ions are essential for triggering membrane fusion and the exocytosis of secretory vesicles in multicellular organisms (4), and membrane-bound extrusomes require fusion with the plasma membrane to discharge (26). Unsurprisingly, extracellular calcium influx modulates extrusome firing across diverse extrusomes in diverse organisms. The molecular underpinnings of Ca^{2+} -modulated exocytosis of trichocysts in *Paramecium*, which have

been reviewed in great detail by Plattner (63), demonstrate that trichocyst matrix proteins bind to extracellular calcium ions that enter the cell in response to stimuli, initiating exocytosis.

6. CELLULAR WEAPONRY ARISING FROM SYMBIOTIC INTERACTIONS

Symbiotic interactions between bacteria and eukaryotes are widespread and have profoundly influenced eukaryotic evolution, cell biology, and ecology (33). Bacterial symbionts can function as both defensive and offensive extrusomes for their eukaryotic hosts. For example, the verrucomicrobial epixenosomes of *B. bacati* and some *Euplotidium* spp. defend the host against predation and are closely related in phylogenies inferred from 16S rDNA sequences, suggesting that these episymbionts may have been laterally transferred between these two hosts (7, 76, 104). Bacteria-derived projectiles can benefit hosts by offensively reducing competitors that lack symbionts from a common environment. In *Paramecium tetraurelia*, evolutionary maintenance of the endosymbionts that produce extrusive R-bodies (i.e., *C. taeniospiralis*) results in a “killer” phenotype that eliminates intraspecific competitors (40, 80, 105). When these killer paramecia encounter strains lacking the symbionts, these strains are eliminated; consequently, competitors are removed, and the survival and reproductive success of the killer strains are enhanced.

The acquisition of pathways that produce cellular weaponry from bacteria is not solely limited to symbiotic interactions between bacteria and their eukaryotic hosts. In fact, horizontal transfer of genes associated with the development of extrusive structures from bacteria that can produce R-bodies may have facilitated the evolution of projectiles in some lineages, such as cryptists (100). Although the ejectisomes of cryptists are produced through vesicular formation in the Golgi apparatus (39) and do not represent symbiotic bacteria like in *Paramecium*, the ribbons that compose these extrusomes contain the nuclear-encoded Tri proteins, which are homologous with proteins that are required for R-body development in *Caedibacter* (100). The pathway that encodes for these proteins (Tri1 in particular) is inferred to have arisen through an ancestral horizontal gene transfer event between an ancestral cryptist and proteobacteria capable of producing R-bodies.

7. CELLULAR WEAPONRY STOLEN FROM PREY ORGANISMS

Extrusomes in eukaryotes can also be acquired and retained by predators that consume prey organisms possessing these specialized structures. For instance, some predators of cnidarians can sequester the nematocysts of their prey and use them for their own defense from predators (19, 23). This form of extrusome acquisition, called kleptocnidae, as mentioned above, has evolved 9–17 different times within animals alone, such as in some ctenophores, flatworms, acoels, and aeolid nudibranchs (19, 23). The evolution of kleptocnidae requires that predators of cnidaria have chemical or physical defenses against the ingested nematocysts, alongside the ability to transport and reposition undischarged (or immature) nematocysts to appropriate tissues (e.g., the cnidosacs of aeolid nudibranchs) (23). Interestingly, kleptocnidae represent a novel functional transition in cellular weaponry; originally offensive extrusomes for predation in cnidarians, the stolen nematocysts, are reutilized for defense in the new animals. The acquisition and evolution of kleptocnidae across animals has been reviewed by Goodheart & Bely (23).

8. MOLECULAR CHARACTERISTICS OF CELLULAR WEAPONS

Our understanding of cellular weaponry, particularly in protists, has been informed primarily by ultrastructural studies using electron microscopy and experimental studies investigating the behavior and survival of cultivated taxa under specific environmental conditions. However, given advancements in molecular biology techniques, particularly those that can be used to understand

uncultivated lineages, we have begun to elucidate the molecular underpinnings of the development, structure, and evolutionary origins of extrusomes across the tree of eukaryotes. Single-cell genomics is paving the way for comparative studies that can improve our understanding of the genes and proteins associated with the development and discharge of different extrusomes in microbial eukaryotes. For example, expanded single-cell genomic and transcriptomic sampling of haptorian ciliates has demonstrated that toxicyst-bearing lineages have significantly more copies and increased expression of genes associated with membrane transport and hydrolysis compared with ciliate lineages that lack toxicysts (51). Similarly, genomics techniques are being used to investigate the mechanisms by which extrusive organelles aid in host invasion and how these structures may have facilitated the evolution of a parasitic lifestyle in different lineages of eukaryotes (e.g., parasitic ciliates and myxozoans) (1, 25, 101). In cultivated and genetically transformable taxa, genetic experiments can be used to elucidate molecular characteristics of extrusome development and discharge. For instance, genetic experiments using mutants of *Paramecium* that have lost or gained function in specific secretory genes have informed of the function of these genes in the development, docking, and discharge of trichocysts (63).

The numerous examples of convergent evolution in extrusomes across distantly related lineages of eukaryotes can be evaluated with more rigor via modern molecular biology techniques, such as single-cell genomics and transcriptomics. These approaches have begun to elucidate whether specific extrusomes in different lineages share a common ancestry, such as the nematocysts of cnidarians and dinoflagellates or the ejectisomes of cryptists and prasinophyte green algae (20, 98). As we continue to study poorly understood protists with modern molecular biology, outstanding questions about the evolutionary relationships of extrusomes as well as the molecular underpinnings behind their development and discharge mechanisms can be addressed with more clarity and confidence.

SUMMARY POINTS

1. Cellular weaponry is widespread across eukaryotes, particularly protists. Increased sampling of poorly studied lineages continually reveals novel extrusomes in these taxa.
2. Convergent evolution of cellular weaponry is common: Morphologically or functionally similar extrusomes have evolved independently among distantly related taxa when subject to similar selective pressures.
3. Homologous extrusomes diverge in structure and function among close relatives, often adapting to lifestyle shifts such as parasitism.
4. Cellular weaponry occurs at multiple organizational levels, from organelles (e.g., nematocysts, trichocysts) to entire cells (e.g., microsporidian spores, haptoglossan gun cells) and multicellular structures (e.g., colloblasts of ctenophores).
5. Extrusomes serve three major functions: (a) prey capture (e.g., harpoons, adhesive threads, toxins), (b) host cell invasion (e.g., microsporidian polar tubes, haptoglossan gun cells, apicomplexan rhoptries), and (c) defense (e.g., trichocysts in ciliates/dinoflagellates, ejectisomes in cryptomonads).
6. Extrusomes are not exclusively endogenous organelles: They can originate from symbiotic bacteria (e.g., R-bodies, epixenosomes) or as weapons stolen from prey (e.g., kleptocnidae).



7. Mechanisms of extrusome discharge are diverse, usually triggered by mechanical or chemical stimuli, with many systems relying on rapid pressure generation or calcium-mediated exocytosis.
8. Advances in molecular biology are revealing the genetic and evolutionary foundations of extrusome development, structure, and discharge across both cultivated and uncultivated eukaryotes.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

AUTHOR CONTRIBUTIONS

M.V.P. and B.S.L. constructed the outline of the review. M.V.P. wrote the initial draft of the manuscript, and B.S.L. built the figures. M.V.P. and B.S.L. edited subsequent iterations of the manuscript. A grant to B.S.L. funded the research.

ACKNOWLEDGMENTS

This review was completed on the traditional, ancestral, and unceded territory of the x^wməθk^wəyəm (Musqueam) people. This work was funded by a grant to B.S.L. from the National Sciences and Engineering Research Council of Canada (NSERC 2025-04268). Perplexity Academic was used to improve the readability of the text.

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