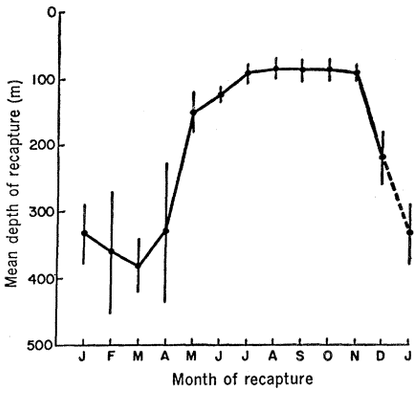

Fig. 2. Mean depth of recapture and 95 percent confidence limits. by months.

of 10° to 17.5°C. It is evident, therefore, that offshore lobsters actively orient to optimum temperature according to season, in contrast to coastal populations that remain localized and subject to wider seasonal extremes and a lower annual mean temperature.

Distribution of tagged lobsters that were recaptured is considered representative of the distribution of the lobster population. Fishermen search for commercial quantities of lobsters throughout the year at depths of from 20 to 700 m, which is a considerably greater range than the 70- to 400-m depth interval from which lobsters for tagging were initially captured.

Twenty-one percent of the recaptured lobsters had moved distances less than 16 km, 58 percent between 16 and 80 km, and 21 percent in excess of 80 km. Ten lobsters had migrated farther than 160 km and one had moved 338 km. The apparent speed of travel ranged up to 10 km/day. Lobsters demonstrating the most extensive migrations were predominantly females. Of the 61 lobsters that had migrated more than 80 km, 41 (67.2 percent) were females —a significantly higher percentage than the 53.6 percent females released. Of the 47 lobsters that had migrated onshore more than 80 km, 37 (78.7 percent) were females—also significantly higher than the percentage of females released. Distance of travel was not related to size; mean carapace length of males (103.9 mm) and females (95.0 mm) that had exceeded 80 km was similar to the mean carapace length of all tagged males (99.3 mm) and females (99.7 mm).

Migration of tagged lobsters into the inshore fishing grounds from the Hudson, Block, Atlantis, and Veatch Canyon areas was generally confined to areas south and west of Cape Cod and included several intrusions into the trap fishery of southern Long Island and one into inner Long Island Sound. Lobsters from Lydonia Canyon, Southwest Georges, and Corsair Canyon shoaled onto Georges Bank. No recoveries were made north of Cape Cod in the Gulf of Maine proper, which suggests little, if any, mixing with coastal stocks in the Gulf of Maine.

The growth rate of lobsters depends on the increase in size at molting and the frequency of molting. The increase in carapace length at molting, for the lobsters that were measured at recapture, averaged 18.7 percent for 53 males and 16.7 percent for 51 females. These growth increments are significantly greater than the 12.5 percent increase for males and females from the inshore Gulf of Maine population reported by Cooper (*3*).

Frequency of molting for offshore lobsters was obtained from 65 recoveries that had been at large for approximately 1 or 2 years. Approximately 97 percent of the lobsters (sexes combined) in the group 60 to 80 mm in size molted annually, 88 percent in the group 80 to 100 mm, and 51 percent in the group 100 to 120 mm. These rates of molting are significantly greater than those reported by Cooper (*3*) for lobsters of similar sizes in the inshore Gulf of Maine population. Additional recoveries are needed to define molt frequency by sex.

The extensive seasonal migrations undertaken by offshore lobsters contrast sharply with the localized movements of coastal stocks. Rate of growth of offshore lobsters exceeds that of inshore lobsters, in terms of both growth increments at molting and frequency of molting. Whether the offshore stocks are genetically distinct from their coastal counterparts has not been firmly established, but it is evident that the shelf edge and slope is a permanent habitat from which small and large-scale excursions are made with seasonal regularity. We hypothesize that the continental slope habitat lacks sufficiently high temperatures during the summer to permit the extrusion and hatching of eggs and the molting and subsequent mating, and that the deficiency is compensated by seasonal shoalward migration to warmer water.

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References and Notes

1. F. E. Firth, *N. Engl. Natur.* **9**, 11 (1940); W. C. Schroeder, *Deep-Sea Res.* **5**, 266 (1958); E. D. McRae, *U.S. Commer. Fish. Rev.* **22**, 1 (1960); B. E. Skud and H. C. Perkins, *U.S. Spec. Sci. Rep.*, No. 598 (1969), p. 1.
2. P. Montreuil, *Sta. Biol. Mar. Grande Riviere Que. Rapp. Annu. 1953* (1954), p. 75; W. Templeman, *J. Biol. Bd. Can.* **1**, 269 (1935); *Newfoundland Dep. Natur. Resources Res. Bull.*, No. 8 (1940), p. 1; D. G. Wilder, *J. Fish Res. Bd. Can. Progr. Rep. Atlantic Coast Sta.*, No. 37 (1947), p. 10; *J. Fish. Res. Bd. Can.* **10**, 371 (1953); *ibid.* **20**, 305 (1963); ——— and R. C. Murray, *Prince Edward Island Fish. Res. Bd. Can. Progr. Rep. Atlantic Coast Sta.*, No. 64 (1956), p. 3; *Fish. Res. Bd. Can. Progr. Rep. Atlantic Coast Sta.*, No. 69 (1958), p. 12.
3. R. A. Cooper, *Trans. Amer. Fish. Soc.* **99**, 409 (1970); D. M. Harriman, *Progr. Rep. on Lobster Tagging—Monhegan, 1951–1952, Maine Dep. Sea Shore Fish.*, No. 5 (1953).
4. J. Bergeron, *Natur. Can.* **94**, 169 (1967).
5. S. B. Saila and J. M. Flowers, *J. Cons.* **31**, 342 (1968).
6. L. A. Walford and R. I. Wicklund, *Serial Atlas of Marine Environment* (American Geographical Society, New York, 1968), folio 15.
7. We thank Bureau of Commercial Fisheries port agents John V. Mahoney, Churchill T. Smith, Fred C. Blossom, Paul Swain, and Dennis Main for invaluable assistance in collecting and reporting tagged lobsters from the commercial fishing fleet.

9 October 1970

Internal Cellular Details of Euglena gracilis Visualized by Scanning Electron Microscopy

Abstract. *Simple washing of live* Euglena gracilis *cleans the pellicle of external coating material. When these cleaned cells are viewed with a scanning electron microscope, organelle outlines can be seen through the thinned pellicle. To view organelles directly, fixed* Euglena *cells are gently broken first or first frozen and then broken as part of their preparation for scanning electron microscopy. Subsequent viewing at magnifications of × 5,000 to × 21,000 reveals organelles, storage granules, endoplasmic reticulum, and nuclear pores, while retaining great depth of focus.*

During the last 5 years scanning electron microscopy has begun to be exploited for the study of soft as well as mineralized biological material (*1*). These studies have revealed many external details of multicellular and uni-

cellular organisms and resolved conflicting theories of the pattern of ciliary motion in protozoa (*2*).

This report describes the use of scanning electron microscopy to view the internal and external cellular architecture of the alga *Euglena gracilis*, Z strain, in such a way that perspective is preserved while detail is observed simultaneously on multiple optical planes. Heretofore such perspective has been obtained, to a limited degree, through the use of freeze-etching followed by visualization with a transmission electron microscope (TEM). Presently, freeze-etching–TEM allows greater resolution of structure (within a narrow optical plane) because of the higher resolution that is obtainable with TEM (*3*).

The fixed *Euglena* cells (*4*) were pulsed with a Vortex mixer for about 30 seconds to fracture 20 to 50 percent of the organisms, and then applied in drops (1 to 2 mm in diameter) to aluminum specimen stubs that had been polished to a mirror finish. After all specimen drops had been applied, the corrugated paper carrier that held the stubs in an upright position was half-immersed in liquid nitrogen to rapidly reduce the temperature of the specimen stubs and thereby freeze the droplets of water containing the samples. The sample stubs, still held in place in the carrier, were rapidly transferred to a lyophilizer and allowed to remain there for several hours longer than the half hour required to complete sublimation of the droplets to dryness. In other cases, Vortex-mixing was omitted, and the unbroken cells were carried through transfer of the stubs to the lyophilizer.

When the sample-containing water droplets were just frozen and evacuation of the lyophilizing chamber was initiated, the lyophilizing chamber was struck sharply with the flat of the hand. This blow coupled with the increasing vacuum caused most of the water droplets to shear off, much as it does in a more orthodox freeze-fracture apparatus. Success of the procedure depends upon the water droplets being just frozen, since it cannot be fractured if the droplet is either partly melted or frozen too solid. A few trial runs usually are enough to establish the appropriate conditions for the particular equipment.

After lyophilization, the stubs were then transferred to a high-vacuum evaporator where they were uniformly

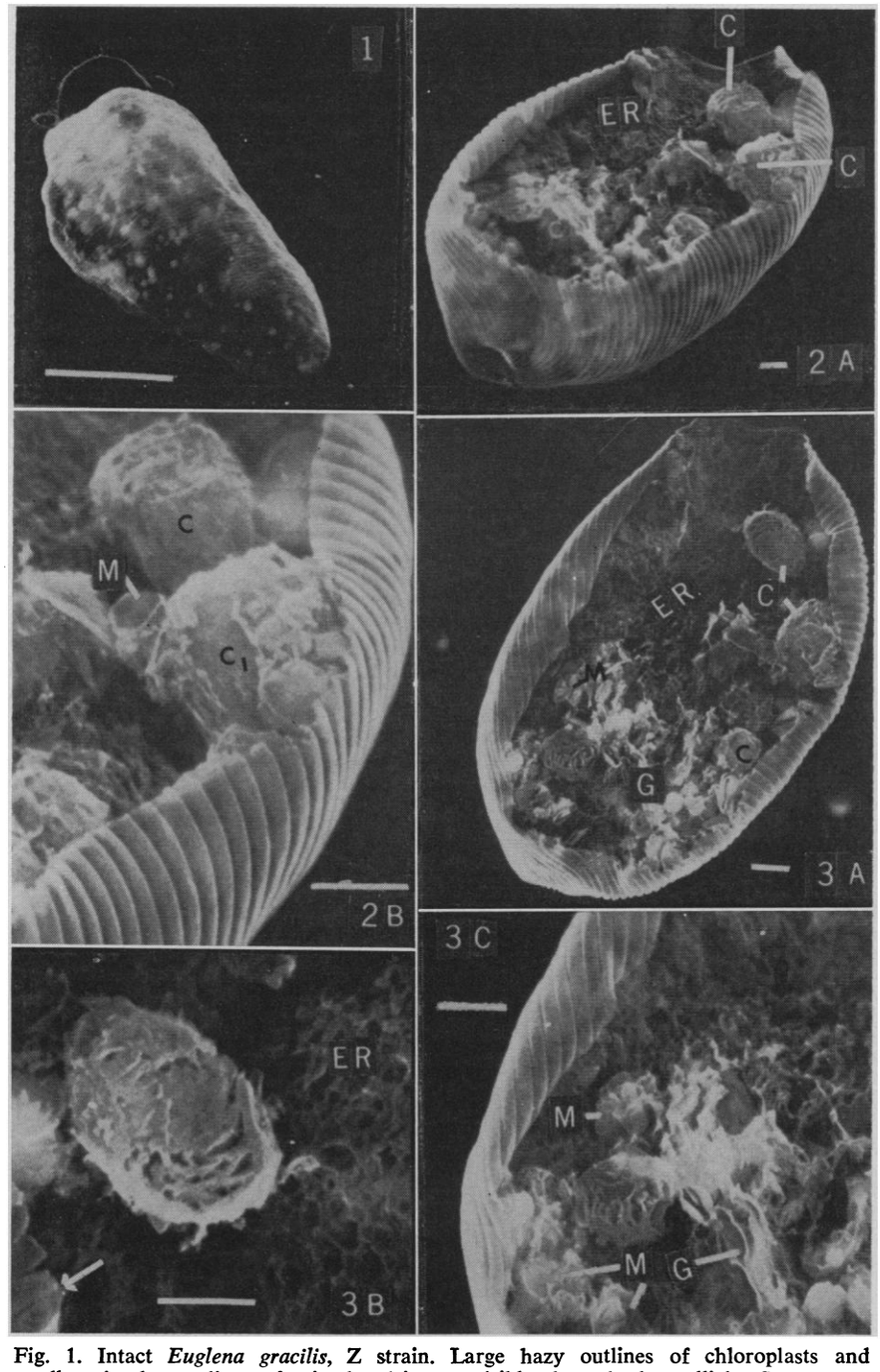

Fig. 1. Intact *Euglena gracilis*, Z strain. Large hazy outlines of chloroplasts and smaller circular outlines of mitochondria are visible through the pellicle. Some mastigonemes can be seen standing free of the flagellum but most of them are loosely wrapped around the flagellum and are not clearly seen at this magnification. Magnification bar equals 10 μm. Fig. 2. Cell broken with Vortex mixer. Magnification bar in this and subsequent figures equals 1 μm. (A) Overall view. Anterior of the cell is in the lower left-hand corner. The flagellum has been shaken off, and the cytostomal depression is revealed. Massive endoplasmic reticulum (*ER*) and chloroplasts (*C*) are apparent. (B) Posterior detail. Chloroplast (*C*) is sheared in half (further details at 0° tilt are seen in Fig. 4B) while chloroplast (C_1) is whole and is covered by endoplasmic reticulum fragments. A broken mitochondrion (*M*) is present. Fig. 3. Cell from Fig. 2 at 0° tilt. (A) Whole cell with entire broken surface exposed. The most anterior marked mitochondrion (*M*) shows typical radial cristae. Presumed Golgi complex (*G*) is indicated. (B) Fractured chloroplast showing lamellar thylakoids embedded in endoplasmic reticulum. The arrow points to a broken pellicular stria. (C) Enlargement of anterior portion of (A). Mitochondrial details (*M*) and presumed Golgi (*G*) are evident.

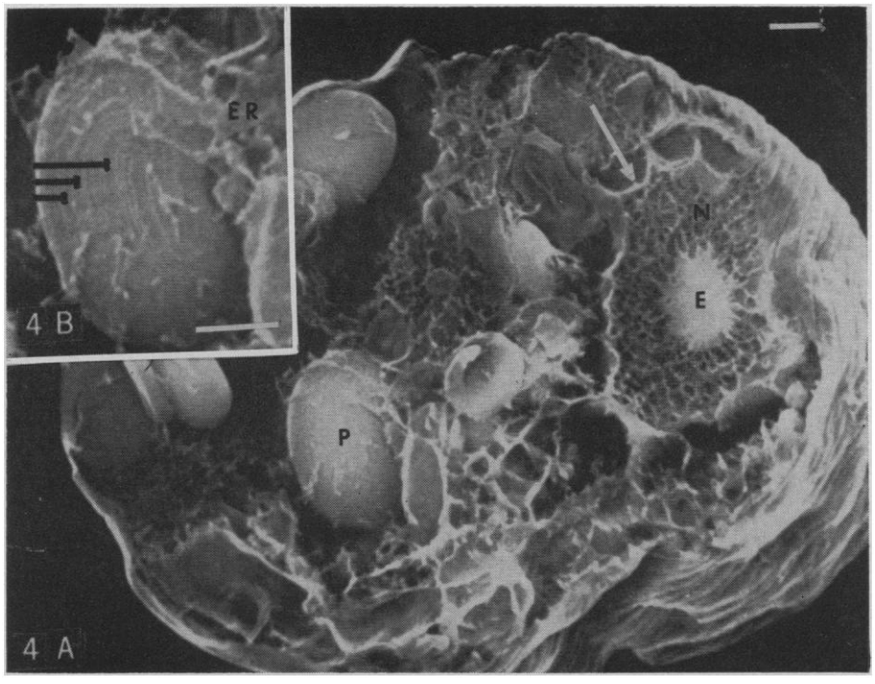

Fig. 4. Dark-grown cell prepared by freeze-fracture and viewed at 0° tilt. (A) Nuclear pores (arrow) and endosome (*E*) are visible. This cell was packed with large paramylon granules (*P*). (B) Enlargement of paramylon granule *P*. The concentric rings (arrows) of the granule are easily distinguished from endoplasmic reticulum overlaid on the granule.

vapor coated (by rotation of the stubs) with gold to a thickness of approximately 200 Å. Coated stubs were stored in a desiccator until they were examined. Specimen stubs were viewed with a Mark IIA scanning electron microscope (SEM) (Cambridge Instrument Co.) set at a 30 kv beam accelerating voltage. Stubs could be viewed continuously for more than 60 minutes without visible alteration of the biological material. Stubs were tilted at an angle of 45° (or in a few cases 0°) to the electron beam.

The short incubation of live cells in distilled water removes much of the polysaccharide external to the algal pellicle and permits pellicular detail and outlines of organelles under the pellicle (*5*) to be seen with ease (Fig. 1). The interrupted helical pattern of the flagellum (Fig. 1) confirms the observations of Jahn and Bovee (*6*) of interrupted helical waves in the locomotory process and renders unlikely Leedale's (*7*) textual contention that flagellar motion is in continuous helical waves. It should be noted that his own photographs and diagrams also confirm the interpretation of Jahn and Bovee (*6*).

Vortex-mixing of the fixed-cell suspension is gentle enough to leave many cells intact and the remainder either with pellicle intact, but devoid of flagella, or with cells broken open. When such cells are viewed at a 45° tilt perspective is evident (Fig. 2A), whereas the flat appearance at 0° tilt makes organelles more easily recognizable. The spongy endoplasmic reticulum virtually fills all portions of the cell not otherwise occupied by the organelles (Figs. 2 and 3). Chloroplasts are easily identified by their size and the lamellar, presumed thylakoid, bands. It is relatively easy to discriminate between endoplasmic reticulum covering a chloroplast (chloroplast C_1 in Fig. 2B) and internal chloroplast lamellae (Fig. 3, A–C).

At least some mitochondria can be identified with certainty by the radial arrangement of cristae which results in an appearance similar to that of an open orange containing several segments (Fig. 3, A and C). Nuclei are particularly easy to identify in cases in which the fracture reveals the endosome (*E* in Fig. 4). Connections between the outer membrane of the nucleus and the main portions of endoplasmic reticulum, and occasionally pores in the outer nuclear membrane (arrow in Fig. 4), are seen. Storage carbohydrate, paramylon granules (*P* in Fig. 4), are readily distinguished from chloroplasts by their smooth appearance save for delicate, concentric surface-rings (*8*). It is not yet possible to positively identify all membranous portions of the cell, but the anterior location of multiple, parallel, flattened lamellae with terminal vesicular appearance relatively clear (Fig. 3A), in at least one place, makes me tentatively consider them to be a portion of the Golgi apparatus.

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References and Notes

1. The most extensive bibliographies on scanning electron microscopy as well as illustrated abstracts that make use of a wide variety of biological material can be found in *Electron Microscopy/1968. Proceedings of the Symposium on the Scanning Electron Microscope—The Instrument and Its Applications*, O. Johari, Ed. (Illinois Institute of Technology Research Institute, Chicago, 1969); *Scanning Electron Microscopy/1970*, O. Johari, Ed. (Illinois Institute of Technology Research Institute, Chicago, 1970).
2. G. A. Hovridge and S. L. Tamm, *Science* **163**, 817 (1969).
3. E. Branton, *Proc. Nat. Acad. Sci. U.S.* **55**, 1048 (1966).
4. *Euglena gracilis* was grown in either defined or undefined media at *p*H 3.6 [H. N. Guttman, in *Analytical Microbiology*, F. Kavanagh, Ed. (Academic Press, New York, 1963), p. 527] at 23° to 26°C under constant fluorescent illumination, or in one case, constant darkness. Cells were gently pelleted centrifugally, resuspended in distilled water, and incubated for an additional 60 to 120 minutes in distilled water. Only actively swimming organisms were chosen for final, gentle centrifugation to ensure selection of normal cells. The pelleted cells were fixed by resuspending them in 0.05*M* PO_4 buffer (*p*H 7.1 to 7.2) containing 1.0 percent (weight/volume) osmium tetroxide and held at 6°C in the dark for 30 to 45 minutes. Excess fixative was removed by aspiration and the precipitated cells were washed at least twice with distilled water. Fixed cells could be stored for several days in a refrigerator before use.
5. The various shapes that can be assumed by the striae, their role in locomotion, and details of alteration in striae pattern, which result in the anterior and posterior termini of the organism, will be described elsewhere (in preparation). The transmission effects, that is, visibility of outlines of organelles that are just under the pellicle, is possibly due to the thinning of the surface coating during washing coupled with the use of relatively high accelerating voltage (30 kv).
6. T. L. Jahn and E. C. Bovee, in *The Biology of* Euglena, D. E. Buetow, Ed. (Academic Press, New York, 1968), vol. 1, p. 84.
7. G. F. Leedale, *The Euglenoid Flagellates* (Prentice-Hall, Englewood Cliffs, N.J., 1967).
8. A publication that appeared after this one was submitted [S. C. Holt and A. I. Stern, *Plant Physiol.* **45**, 475 (1970)] contains transmission electron microscopic pictures of freeze-fractured paramylon granules showing the concentric layering. The authors suggested that the granules are covered with a rough-textured nonstriated outer layer, but my pictures (for example, Fig. 4) suggest that the concentric layers extend to the surface of the granules and that the nonstriated "outer layer" is probably endoplasmic reticulum which typically sheathes the granules.
9. I thank Ronald Wibel for excellent technical assistance.

17 July 1970