PRELIMINARY OBSERVATIONS OF FEEDING IN THE PSAMMOBIOTIC CILIATE *TRACHELORAPHIS*

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(Received 19 October, 1983)

Abstract. Feeding on large particulates in *Tracheloraphis* species has been observed in living specimens to occur along the nonciliated glabrous stripe rather than at the narrow anterior end. The absence of specialized oral kinetids in this region indicates that feeding occurs by some form of membrane infolding. Organisms were cultured using cooked egg yolk as the sole source of added nutrient, enabling the observation of the feeding process in these relatively primitive ciliates.

1. Introduction

The genus *Tracheloraphis* Dragesco is a primitive genus in a group of psammobiotic ciliates (Corliss, 1975; Corliss and Hartwig, 1977) within the newly erected class Karyorelictea (Small and Lynn, 1981, 1983). These organisms are found widely distributed in both marine and estuarine habitats. A major distinguishing characteristic of the genus is the presence of a nonciliated glabrous stripe, one to several kinetal rows in width, which extends the complete length of the cell in most of the presently described species. In *Tracheloraphis dogieli* (up to now, the only *Tracheloraphis* species whose cytoarchictural ultrastructure has been characterized with transmission electron microscopy), a single row of nonciliated paired kinetosomes are found on either side of the stripe (Raikov *et al.*, 1975). In protargol-stained specimens of other species, barren (without cilia) paired kinetosomes also may be seen.

Direct observation of feeding in tracheloraphid ciliates is undescribed. The assumption of apical feeding has been based on the presence of cytologically unknown inclusion bodies grouped together at the anterior end and of long flexible cilia around the invaginated anterior end of some but not all species (Dragesco, 1960). Their somewhat *Lacrymaria*-like shape may have also influenced the notion of similar modes of feeding by members of the two genera. In fact, observations of protargol-stained whole specimens by Dragesco (1963) and by us (unpublished), of the electron micrographs of *T. dogieli* (Raikov *et al.*, 1975), and of Raikov's (see Raikov and Kovaleva, 1968) published drawings of other nonprotargol stained specimens from Eurasia have yielded neither consistent nor sufficient information to state that a true ciliate cytostomal apparatus does or does not exist at the apical end of tracheloraphids (see Small, 1983, for further clarification of this important point). This anterior region has, nevertheless, been described as the oral area (Dragesco, 1960; Raikov *et al.*, 1975). A few instances of large particulate foodstuffs in presumed food vacuoles have been noted: for example, diatoms (Raikov and Kovaleva, 1968), and a copepod, copepod egg case, and a foraminiferan (Hartwig, 1973). No explanation has been given for their

Origins of Life **13** (1984) 229-234. 0302-1688/84/0133-0229$00.90.

presence or for their mode of entry into the cell. We have observed large inclusions such as centric and pennate diatoms, cyanobacterial filamentous trichomes, and one large undetermined ciliate. These observations suggest that feeding might occur at a site away from the narrow anterior end. Feeding in these organisms might occur along the glabrous stripe by some ingestatory process based on the presence of these very large particulates enclosed in presumed food vacuoles and the presence of only a single cell membrane enclosing the glabrous stripe.

Using a modification of a cooked egg yolk medium devised by one of us (J.G.), *Tracheloraphis* species were grown for the first time in culture. The use of this culture technique enabled us to observe the feeding process.

2. Methods

Tracheloraphis species were isolated from sediments at three sites in the mid-Atlantic region: two estuarine strains from the Chesapeake Bay – Point Lookout, MD and Gloucester, VA – and the other derived from full-strength sea-water from Chincoteague, VA. Initially, the tracheloraphids used were separated from their sandy sediments by means of the Uhlig (1966) technique, as modified by Fenchel (1969), and later by Boynton and Small (1983). A 35 μm nylon monofilament mesh screen (Nitex, from Tobler, Ernst, and Traber, Inc.), separated the original sand-water mixture from the water into which the ciliates moved via the above separation technique. Otherwise, the derived organisms were concentrated by mouth pipette, picking them up from petri dishes that contained small amounts of the sand-water mixture diluted with water from each of the collection sites. Both of these techniques required the use of a binocular compound stereo-microscope (Bausch and Lomb, Inc.) whose magnification ranged from 20–90 diameters. Following extraction and isolation, the ciliates were starved for 24 hours before transfer by mouth pipette to the culture medium.

The culture medium consisted of 0.45 μm millipore-filtered water from each of the collection sites to which streptomycin-SO_4 and penicillin-g were added so that a final concentration of 100 mg/l was achieved for each (Kohlmeyer and Kohlmeyer, 1976). Nutrient was supplied by the addition of very small amounts of freshly boiled egg yolk (Balamuth and Sandza, 1944). A few particles were added to the culture dishes every 5–7 days. Subculturing was effected after a 2–4 week acclimation period.

Light microscopy was carried out using a Nomarski interference microscope (Zeiss, Inc.). Photomicrography of the feeding process was accomplished by using a 35 mm attachment camera back (Exacta) on a trinocular nosepiece. In conjunction with a substage microflash equipped with a 1/2000 second flash xenon light source (Zeiss, Inc.), 200 ASA high speed color negative film (Kodak Ektachrome) was used.

3. Results

Feeding on egg yolk particles occurs 1–3 hours after starved organisms are transferred to the medium. Feeding takes place not at the anterior end but mid-ventrally along the

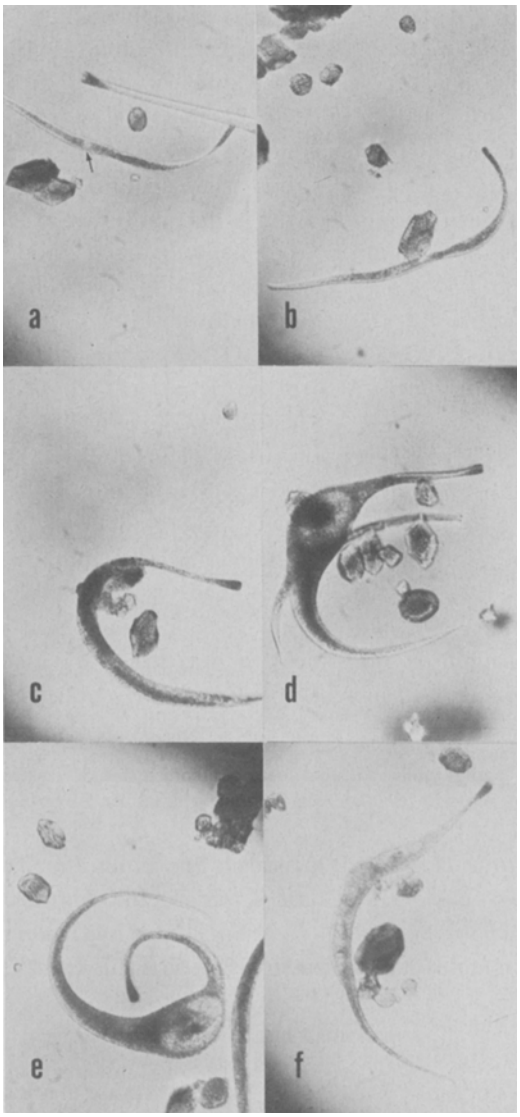

Fig. 1. Feeding in *Tracheloraphis* species. a. Two freshly isolated tracheloraphid ciliates. Arrow indicates single nuclear complex. b. Ciliate 'rubbing' and twisting its body along the surface of an egg yolk particle, with the glabrous stripe touching the particle surface. c. Onset of particle ingestion. d. Partially ingested egg yolk particle. The presumptive food vacuole has formed, and the body has become enlarged at the site of ingestion. e. Particle fully enclosed in the food vacuole. f. Ciliate with multiple food vacuoles containing egg yolk particles.

glabrous stripe (Figures 1 and 2a-f). In forms with a single nuclear complex, feeding usually occurs posterior to that complex. No observations have been made of the process in cells that have multiple nuclear configurations.

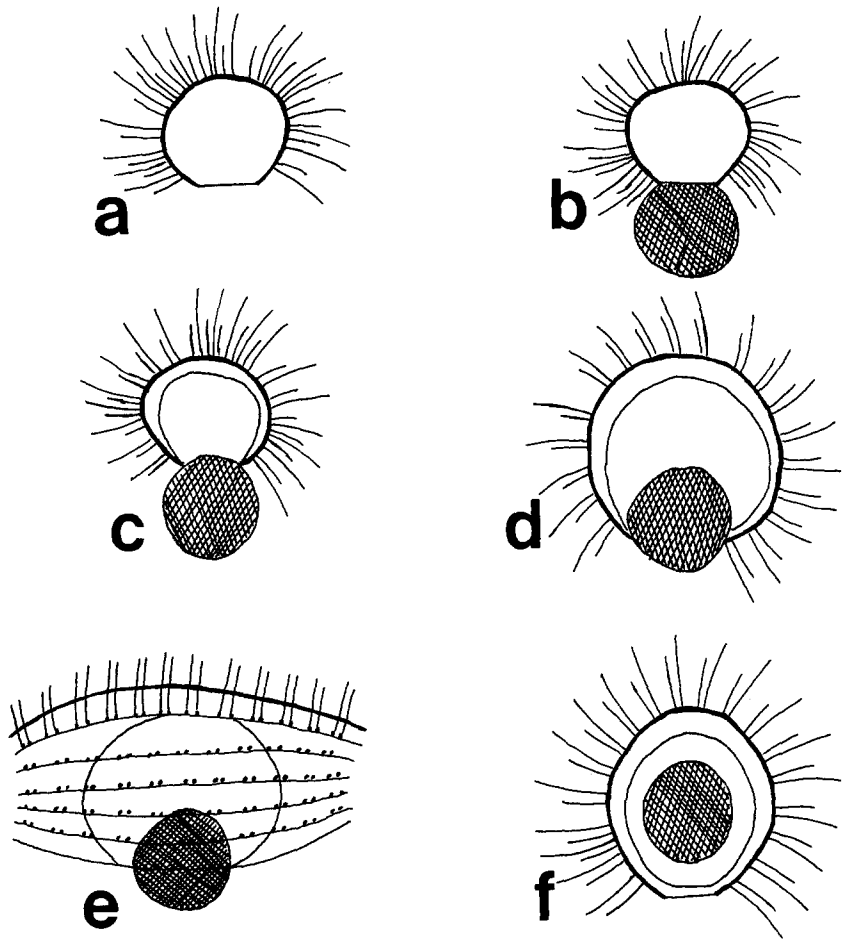

Fig. 2. Diagrammatic representation of the feeding sequence for *Tracheloraphis* species based on living specimens. a. Cross-section of a hypothetical tracheloraphid ciliate showing the nonciliated glabrous stripe (lower surface). b. Initial contact with the particle at the ingestatory site. c. Onset of particle ingestion with the organism attached to the particle. d. Partial ingestion of the particle showing the formation of the presumptive food vacuole and the enlargement of the body. e. Lateral view of the organism showing particle ingestion and presumptive food vacuole formation. f. Particle completely enclosed in a food vacuole, and reformation of the glabrous region.

Prior to feeding, the ciliates may or may not 'bump' the particle with the anterior end. This activity, if present, is followed by an action whereby the organisms appear to twist the glabrous stripe from one side to the other and, in the process, make contact with the particle surface (Figure 1b). The particle may also touch the somatic cilia of the somatic kineties on either side of the stripe. The organism so orients its body that it is atop the particle with the glabrous stripe flush against the particle surface (Figure 1c). During this time, a presumptive food vacuole is being formed and may ultimately achieve a size much larger than the food particle, prior to the actual ingestion of it

(Figure 1d). The ciliate appears to 'push' against the piece of food that is lying on the petri dish bottom until the particle is fully enclosed in a food vacuole. Then the ciliate glabrous stripe appears to be continuous again (Figure 1e).

Ingestion of large particulates results in the deformation of the body at the site of ingestion with the anterior and posterior regions not visibly affected. The ciliates do not appear to stop feeding after the ingestion of a single bit of egg yolk, as proven by the presence of large numbers of food vacuoles in some organisms (Figure 1f). The particles found in any one vacuole may vary in both size and number, with a large vacuole containing from one to six particles of varied sizes.

Observations have been made of the organisms expelling diatom frustules, after which the ciliates return to their original shape. They have not been seen to expel the egg yolk particles. It should be noted that these organisms lack any form of organized cytoproct (Raikov *et al.*, 1975, and our own observations based on stained preparations). We do not yet know how elimination of particulate waste substances occurs in these psammobiotic protozoa.

4. Discussion

Feeding on large particulates in *Tracheloraphis* species occurs along the nonciliated glabrous stripe rather than at the narrow anterior end. We hypothesize that this phagotrophic process may be a form of membrane infolding, which differs from phagocytosis in rhizopod amoebae in that no pseudopodial food cup is formed (Marsot and Couillard, 1978). In the absence of transmission electron micrographic evidence, we cannot comment at this time on the exact mechanism by which the food vacuole forms from the cell membrane itself or from precursory flattened vesicles added to the site of ingestion. Unlike the more evolutionarily advanced ciliate groups, true oral dikinetidal architecture is absent (see Small, 1983) nor is there a single site of ingestion. Feeding appears to be possible anywhere along the glabrous stripe. This mode of ingestion has not been demonstrated in any of the other described karyorelictean ciliate genera (see Fenchel, 1968) that possess similar glabrous striped surfaces. Such studies should be made to more completely understand the broader significance of these preliminary observations of ours.

At present the taxonomy of these organisms is in a state of anarchy due to the absence of decently stained organisms and subsequently detailed comparative analyses of their cytoarchitecture. The cells are highly contractile and fragile (Dragesco, 1960). In addition, various nonkinetosomal cytoplasmic inclusions (e.g. various presumed extrusomes), are stained – when the protargol silver technique is used – making it impossible to accurately determine kinetal organization, believed to be crucial to systematic assignment (Small and Lynn, 1983). Many of the known species are thus far poorly studied cytologically and identifications are based on either live observations or from insufficiently stained preparations. Prior to this study, no one has successfully cultured any species of the genus.

Dragesco (1960) suggested that toxicysts (formerly called toxic trichocysts) were

involved in food capture. However, in our stained and sectioned preparations there has been no supportive evidence for this conjecture. Dragesco (1962) further studied predation by *Dileptus* and other rapacious haptoreans, showing that toxicysts are involved in prey capture in those cases. However, no cytological evidence is available to support the notion that *Tracheloraphis* is rapacious. Lysis of the egg yolk particle does not occur until long after the particle has been enclosed in the food vacuole. Like *Dileptus*, food vacuole formation appears to occur during particle ingestion. Exactly how tracheloraphids capture copepods and other large prey is uncertain. Perhaps these prey are already moribund. The long anteriormost cilia may be sensory, although there is no evidence to support this, other than the occasional contact with food particles prior to ingestion.

Preliminary, yet direct, observations of macrophagous ingestion in *Tracheloraphis* species has been observed, and the uniqueness of the process, as far as we know it, has been critically appraised. This paper offers the suggestion that feeding is not prostomeal in at least the organisms we have thus far observed. Further, the glabrous stripe appears to be the site for the ingestion of food. In the absence of a distinct cytostome in the glabrous region, some form of folding-in of the glabrous zone membrane must indeed *initially* take place. Further studies of the feeding to explain more mechanistically the role of membrane precursors in the ingestatory process at the electron microscopic level are under way by one of us (S.E.L.).

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