

CILIARY MOTION IN *PARAMECIUM*

A Scanning Electron Microscope Study

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INTRODUCTION

The scanning electron microscope (SEM) provides an ideal system for studying the three-dimensional surface structure of biological materials. Our previous work on the protozoan *Opalina* showed that instantaneous fixation, combined with critical point drying, faithfully preserved the pattern of ciliary coordination and the form of ciliary beat for scanning electron microscopy (1, 2).

I report here new results using these techniques to study ciliary motion in *Paramecium multimicronucleatum*. In addition, I have compared the suitability of freeze-drying vs. critical point drying as a method for preparing *Paramecium* for the SEM.

MATERIALS AND METHODS

P. multimicronucleatum,¹ grown in Vegemite medium (3), were washed in an equilibration medium (4) containing 1 mM CaCl₂, 2 mM KCl, and 5 mM Tris buffer (pH 7.0). Cells swimming in a small volume of equilibration medium were fixed instantaneously by rapid addition of a large volume of 2.7% OsO₄ and 2.3% HgCl₂ (modified after Parducz [5]). After 10–15 min in fixative at room temperature, the cells were washed in distilled water. To freeze-dry specimens, small drops of cells on aluminum “spoons” were plunged into a beaker of liquid propane which floated on liquid nitrogen. Frozen specimens were quickly transferred to a liquid nitrogen-cooled cold stage in a vacuum chamber, and the chamber was pumped down. After drying was completed (overnight), the chamber was gradually warmed to room temperature and the cells were removed. Dry cells were placed on specimen stubs, uniformly coated with gold-palladium, and viewed in a JSM-U3 SEM.

¹ Isolated by Dr. Ian Stevenson in 1969 from the Murrumbidgee River near Canberra, Australia.

Critical point drying was used to prepare other paramecia for the SEM. These *P. multimicronucleatum*² were grown in baked lettuce medium (3). The washing and fixation procedures were carried out as described above. Specimens were dried by the critical point method of Horridge and Tamm (1),³ coated with gold-palladium, and viewed in a Cambridge Mark 2A SEM.

RESULTS

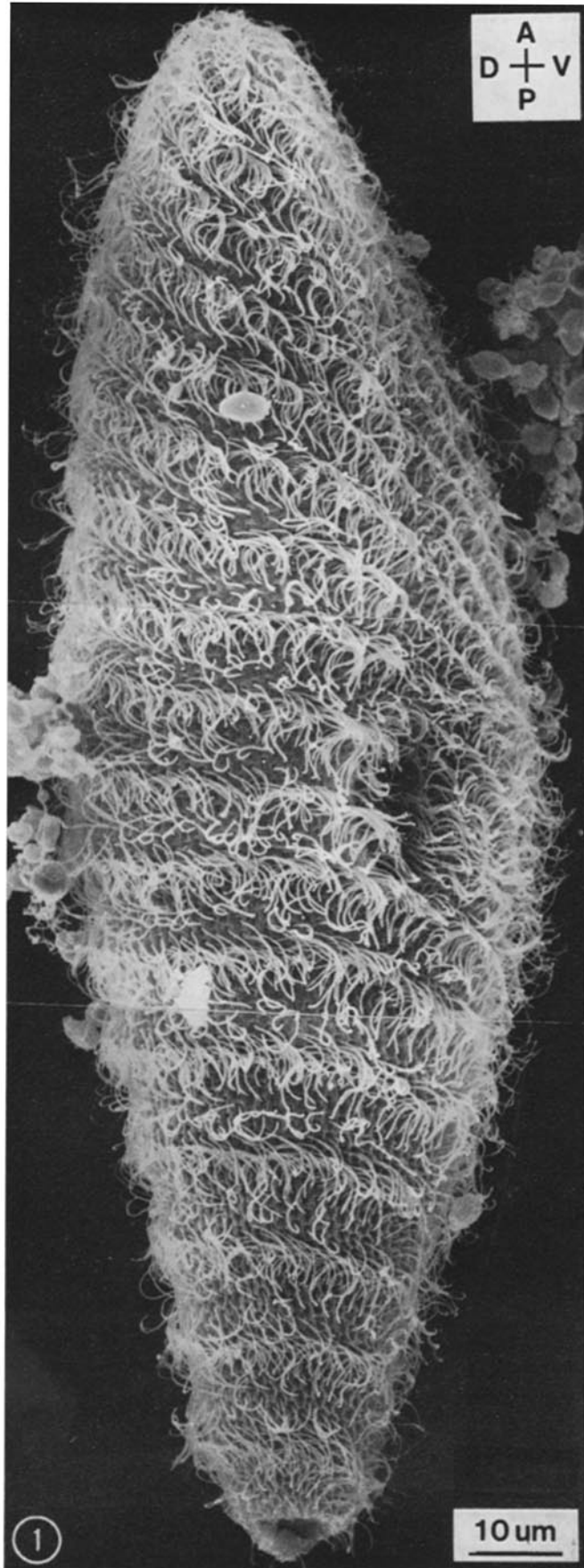
In the forward-swimming paramecia, adjacent somatic cilia lying in a direction slightly oblique to the cell's anterior-posterior axis beat with regular phase differences (metachronally), while the beat cycles of cilia in rows perpendicular to this direction are synchronized. Cilia in consecutive stages of the beat cycle follow each other from front to rear, i.e., “each cilium successively assumes the position of its posterior rather than its anterior neighbor” (5). The result is a series of metachronal waves of ciliary activity which travel from left posterior to right anterior over the surface of cells fixed during forward swimming (Figs. 1–3).

The form of bending during a complete beat cycle of a somatic cilium can therefore be reconstructed from the micrographs by following the positions of successive cilia as one moves from anterior to posterior through a single metachronal wave. For convenience, we may start with the low-

² Syngen 2, stock 46, kindly supplied by Dr. T. M. Sonneborn.

³ The critical point apparatus used in this study was built by Dr. Colin Muir, Zoology Department, University of St. Andrews, St. Andrews, Scotland. It is now commercially available from the Monroe County Tool Company, Ellettsville, Indiana.

FIGURE 1 Scanning electron micrograph of a freeze-dried *Paramecium* fixed during forward swimming. Metachronal ciliary waves travel from posterior to anterior. Note the opening to the mouth on the ventral side, and the polygonal nature of the cell cortex. Contaminating yeast cells from the culture medium are evident. A-P, anterior-posterior axis; D-V, dorsal-ventral sides.



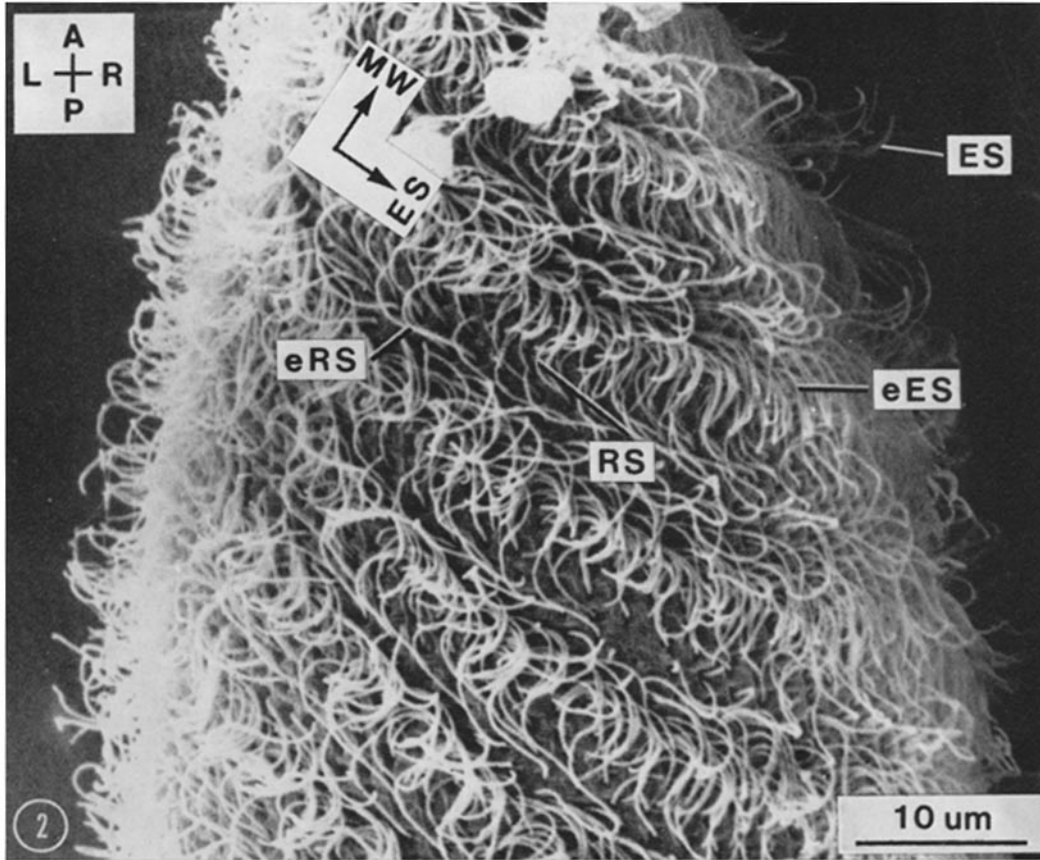


FIGURE 2 Anterior dorsal surface of a freeze-dried *Paramecium* fixed during forward swimming. Metachronal waves travel from left posterior to right anterior (*MW* arrow). The effective stroke of all cilia takes place from a left anterior to a right posterior direction, out of the plane of the micrograph (*ES* arrow). *ES*, cilia in the effective stroke; *eES*, cilia at end of the effective stroke; *RS*, cilia in the recovery stroke; *eRS*, cilia at end of the recovery stroke. *A-P*, anterior-posterior axis; *L-R*, left-right sides.

lying cilia which point in a right backward direction almost parallel to the wave fronts. These are cilia at the end of the effective or power stroke (*eES* cilia, Figs. 2, 3). Moving posteriorly, cilia are encountered which progressively curve or rotate counterclockwise (as viewed from above) close to the body surface. These are cilia in the recovery stroke (*RS* cilia, Figs. 2, 3). At the end of recovery phase, and preparatory to the next effective stroke, cilia have assumed an S-shaped position, directed obliquely forward to the left (*eRS* cilia, Figs. 2, 3). Between these cilia poised to initiate the effective stroke and those which have already completed it, cilia in an erect position can be seen. These cilia have a reversed S shape and are fixed in the act of performing the effective stroke (*ES* cilia, Figs. 2, 3).

Thus, the effective stroke takes place from a left forward direction to a right backward direction, in a plane which is almost parallel to the metachronal wave front. Since the waves travel anteriorly, the pattern of ciliary coordination is approximately dexioplectic, according to the terminology of Knight-Jones (6). The subsequent recovery phase of the beat cycle is a counterclockwise gyration, close to the cell surface, and out of the plane of the effective stroke. The complete beat cycle is therefore an asymmetric movement in three dimensions (Fig. 4).

This description of the form of ciliary beat and the pattern of ciliary coordination holds true regardless of whether cells were dried by the critical point method (Fig. 3), or by freeze-drying (Figs. 1,

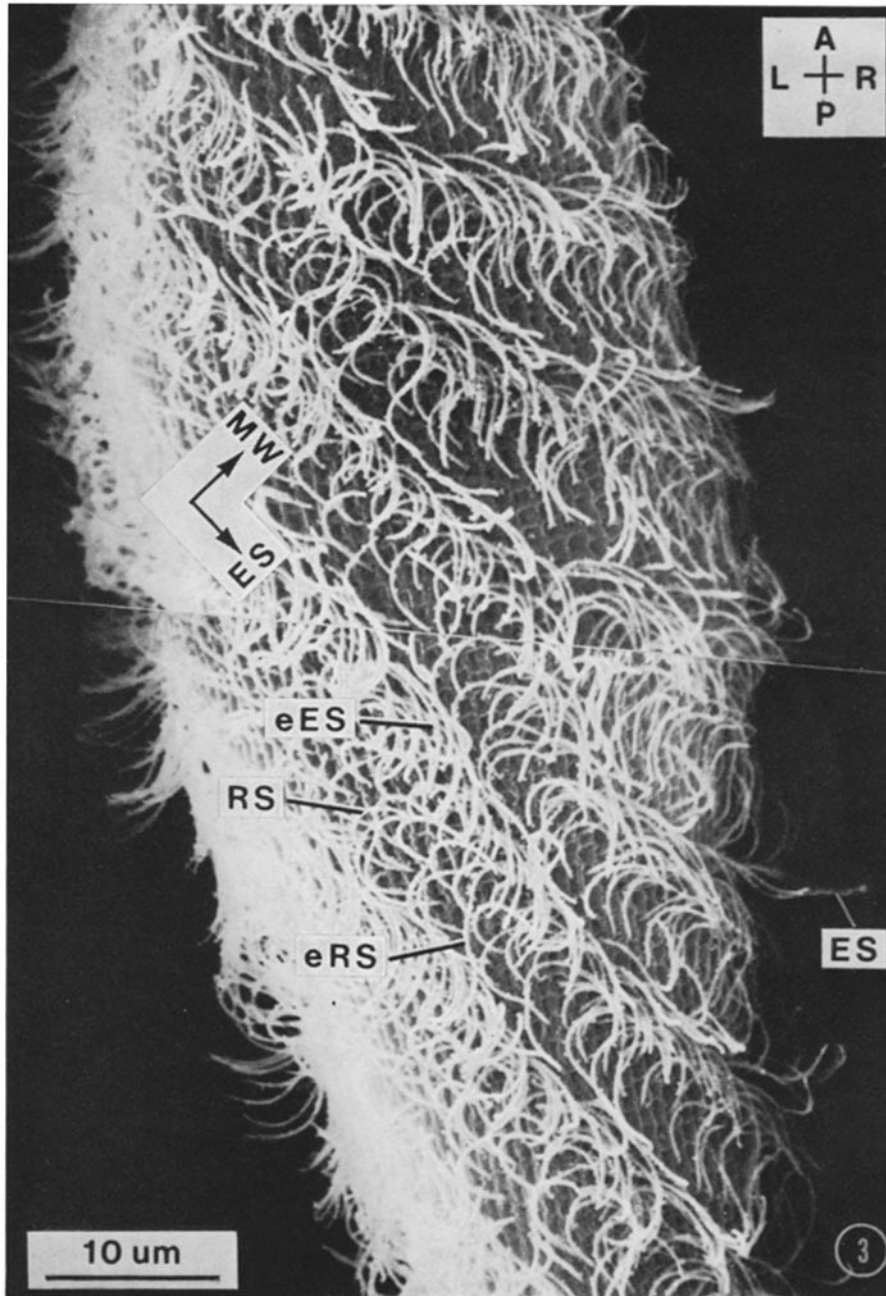


FIGURE 3 Middorsal surface of a critical point-dried *Paramecium* fixed during forward swimming. Arrows and letters have the same meaning as in Fig. 2. The “bumpy” appearance of the cilia is due to contamination from the medium.

2). Therefore, both drying procedures preserve the three-dimensional pattern of ciliary movement equally well. However, I prefer to use critical point drying, since this method is quicker and less costly than freeze-drying.

DISCUSSION

My findings are almost identical with those of Parducz (5), who used instantaneous fixation combined with light microscopic visualization of the

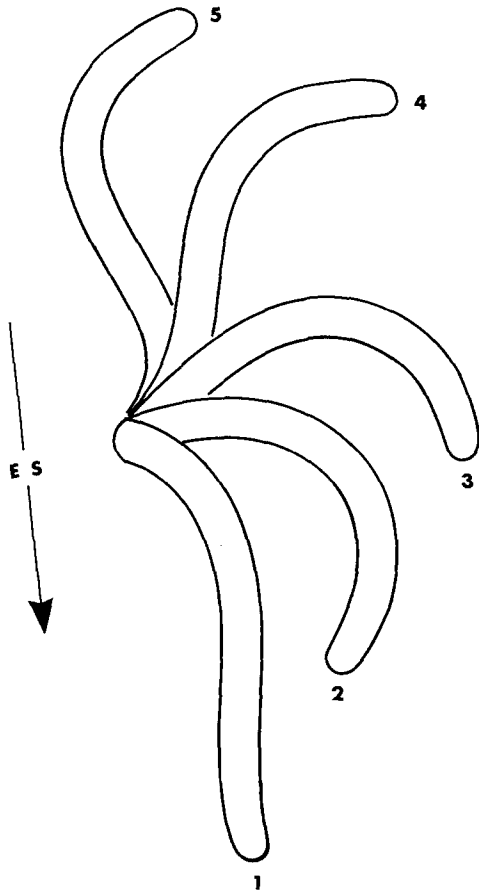


FIGURE 4 Summary of the form of beat of a single somatic cilium of *Paramecium*, as viewed from above. Numbers refer to consecutive stages in the beat cycle: (1) at end of the effective stroke; (2-4), counterclockwise rotation during the recovery stroke; (5), at end of the recovery stroke and preparatory to the effective stroke. The effective stroke (ES arrow) takes place from position five to position one, out of the plane of the paper.

ciliary beat pattern. Recently, Kuznicki et al. (7) presented cinemicrographical evidence that the cilia of *P. multimicronucleatum* beat with a traveling helical wave from base to tip, rather than with a back and forth motion as described here. For technical reasons, these authors published only photographs of cells swimming in medium of high viscosity. However, they claimed that a helical form of beat also occurs under conditions of normal viscosity, where "motion pictures with adequate resolution are almost impossible to obtain" (7).

More recently, Machemer (8) has used flash photography and Nomarski interference-contrast

optics to study the form of beat in paramecia swimming under different viscosity conditions. At normal viscosity the form of beat was similar to that described here for fixed material. Interestingly, at high viscosity, comparable to the conditions used by Kuznicki et al. (7), the form of beat changed into a traveling helical wave. Using similar methods, I have confirmed Machemer's findings at normal viscosity.⁴ It therefore appears that the form of beat in *Paramecium* is viscosity dependent, and that the instantaneous fixation technique is not causing artifacts. Indeed, our previous work on *Opalina* showed a similar form of beat in living vs. fixed material, confirming the validity of instantaneous fixation as a method for faithfully preserving the pattern of ciliary activity (2).

In conclusion, the ability to fix and preserve ciliary motion in *Paramecium* for the SEM should allow a correlated scanning and transmission electron microscopic analysis of the structural mechanisms controlling beat direction in this ciliate, as has already been done in *Opalina* (2). The availability of cortical and behavioral mutants of *Paramecium* (9, 10) offers further advantages for elucidating structure-function relationships in ciliary movement.

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