Protoplasma (1992) 166: 1–7

PROTOPLASMA

Flagella of a chrysophycean alga play an active role in prey capture and selection

Direct observations on *Epipyxis pulchra* using image enhanced video microscopy

R. Wetherbee[1,*] and **R. A. Andersen**[2]

[1] School of Botany, University of Melbourne, Melbourne, Victoria, and [2] Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine

Received January 15, 1991
Accepted June 20, 1991

Summary. The flagella of the pigmented alga *Epipyxis pulchra* (Chrysophyceae) were observed with image enhanced video microscopy to play an active role in gathering, physically seizing and selecting prey prior to phagocytosis. Vegetative unicells of this sessile, freshwater species possess two structurally and functionally distinct flagella, both active in feeding. During prey gathering the long flagellum, which is adorned with stiff hairs, beats rapidly to direct a strong water current towards the cell while the short, smooth flagellum moves very little. When a potential food particle is drawn by the current to contact the flagellar surfaces, the long flagellum stops beating and positions itself, in concert with the short flagellum, to seize the prey between them. Both flagella then briefly rotate the prey before selecting or rejecting it. If rejected, the particle is discarded by the coordinated activity of both flagella. If selected as food, the prey is held in place until a complex collecting cup emanates out from a position near the basal bodies and engulfs it. The cup plus enclosed food particle, now a food vacuole, is then retracted back to the cell proper.

Keywords: Prey capture; Flagella; Chrysophyceae; Video Microscopy.

Introduction

Phytoflagellates from several algal classes are known to be important contributors to aquatic primary productivity, although ecologists have largely disregarded their role as consumers. Recent evidence from field studies shows that mixotrophic phytoflagellates, which are capable of both photosynthesis and phagotrophy as well as ingesting dissolved organic matter, can have a major grazing impact (over 70% reduction in some cases) on populations of picoplankton (for review, see Sanders and Porter 1988). For some pigmented flagellates from the division Chrysophyta, phagotrophy rather than phototrophy is known to be the major nutritional mechanism supporting growth, these organisms commonly phagocytizing food particles that are either non-living (e.g., detritus, fecal material, etc.) or living (e.g., bacteria, small algae, or even cells of their own kind) (Pringsheim 1952, Cole and Wynne 1974, Sanders and Porter 1988, Andersson et al. 1989, Sanders et al. 1990).

Although observations of prey capture by chrysophycean flagellates are found in the literature (e.g., James-Clark 1867, Cienkowsky 1870, Saville-Kent 1880, Stokes 1885, Pringsheim 1952, Aaronson 1973, Cole and Wynne 1974), details of the actual event (i.e., seizure and engulfment) are largely obscure, perhaps due to the involvement of flagella and the speed at which entrapment occurs. Flagella are highly complex organelles capable of numerous activities, particularly in relatively simple unicellular algae. In addition to providing motility, flagella may be involved in cell–cell recognition during mating (for review, see Goodenough and Adair 1989) and in complex cellular movements such as gliding and phototaxis (for reviews, see Bloodgood 1982, Foster and Smyth 1980). To date, the role of flagella in prey capture has been reported as a passive participation, i.e., creating a water current that carries the food particles to the cell.

We now report that in *Epipyxis pulchra* Hilliard et Asmund, flagella not only attract food particles to the cell, but are actively involved in physically seizing prey

* Correspondence and reprints: School of Botany, University of Melbourne, Parkville, Vic. 3052, Australia.

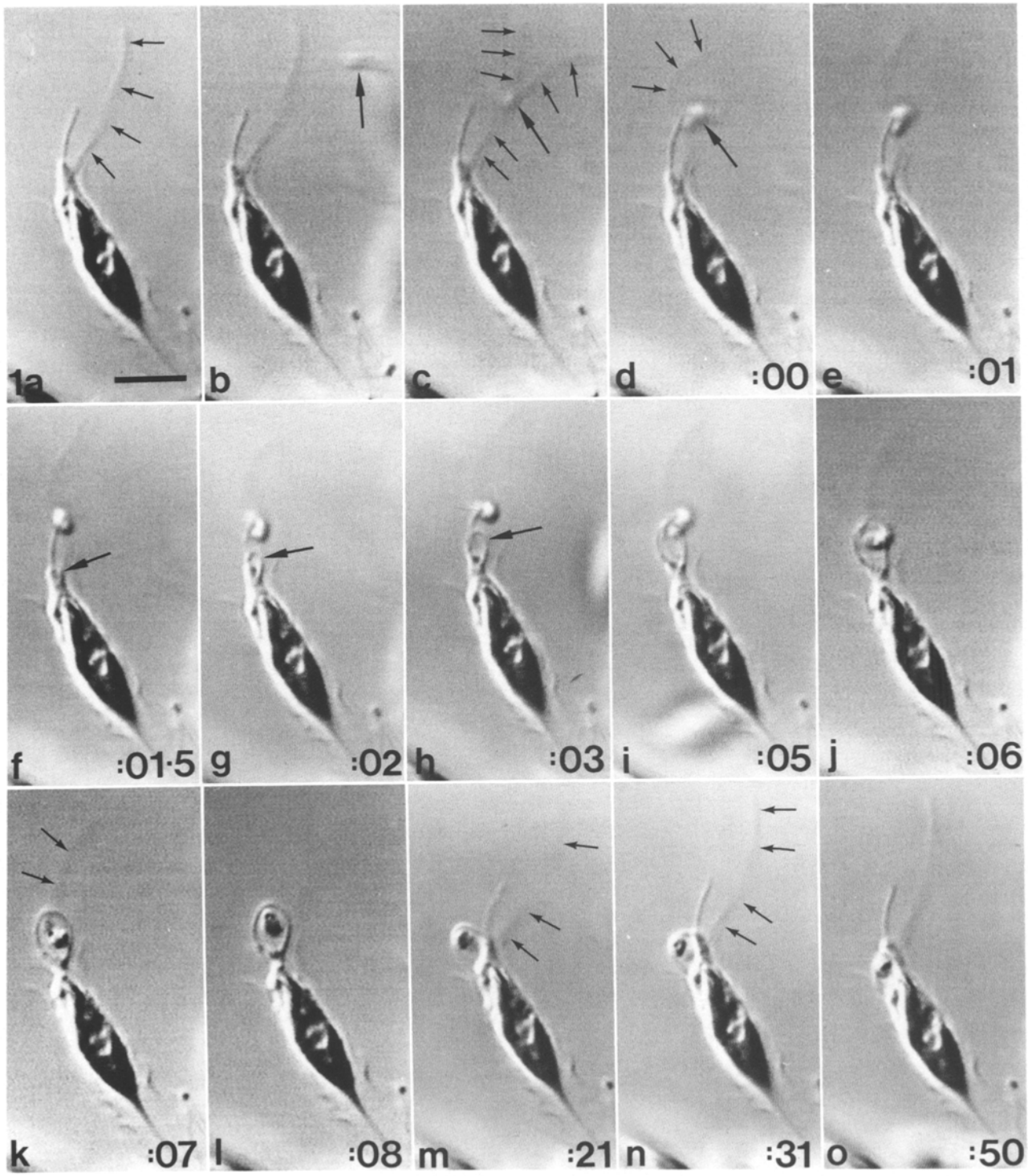


Fig. 1. Images frozen from a video sequence showing the process of prey capture in *Epipyxis pulchra*. Numerals on the bottom right of each figure refer to the time sequence in seconds. Small arrows illustrate the position of the long flagellum during prey attraction (**a, b,** and **m–o**), its contortion during the seizure of prey (**c–i**), and its placement into the cup (**j** and **k**). Capture begins when the prey (large arrow, **b–d**) is first seized by the flagella (**d**) and then briefly rotated (**d–f**). The feeding cup develops within 1–2 s (large arrow, **f–i**) and becomes a food vacuole once the prey is engulfed (**j** and **k**). The food vacuole is then slowly retracted back into the cell (**j–o**). Bar: 10 μm

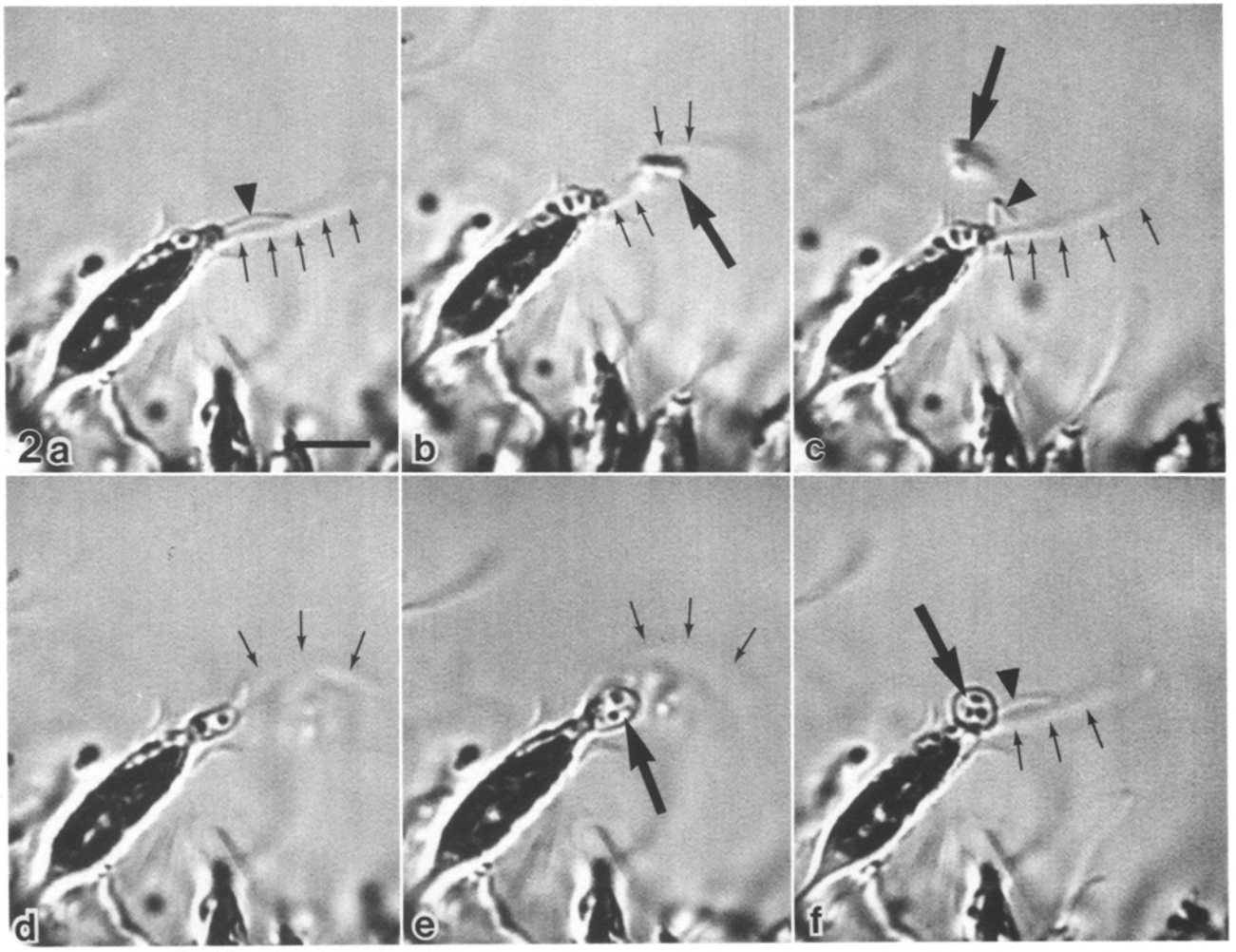


Fig. 2. Frozen images taken from a video sequence showing the capture and subsequent rejection of prey (**a–c**) followed by the capture and acceptance of several prey in rapid succession and their placement into a single cup (**d–f**). The long flagellum (small arrows) contorts sharply (**a** and **f**) during prey seizure. If prey (large arrow, **b** and **c**) is rejected the short flagellum (arrowhead, **c**) bends in a precise and predictable manner to effect release. Occasionally flagella capture several prey and quickly deposit them into the same cup (**d–f**). **d** and **e** Two planes of focus taken at virtually the same time showing 4 small prey within a single cup that becomes a food vacuole (large arrow, **f**) while the long flagellum (small arrows) and short flagellum (arrowhead) resume their normal beat (**f**). Bar: 7 μm

prior to their engulfment. In addition, we suggest flagella have a recognition role, as unwanted cells or particles are quickly discarded after only brief contact with the flagella.

Materials and methods

The culture of *Epipyxis pulchra* was from the chrysophyte collection of the second author (RAA) and are housed at the Provasoli-Guillard Culture Collection at the Bigelow Marine Laboratories, West Boothbay Harbor, Maine. Cells were grown in steam sterilized soil water (25%) at 15 °C with a 15:9 h light:dark cycle. Cells normally feed during the dark period. For light microscopy, cells were mounted on glass slides sealed with Valap (contains equal weights of paraffin wax, lanolin, and vaseline) and examined with either a Zeiss Photomicroscope III or a Nikon Optiphot-pol microscope using Nomarski differential interference contrast optics with a × 100, oil-immersion lens. Increased magnification came from passing the image through a × 4 projector lens before collection by a Hamamatsu C2400 video camera system incorporating a camera head and separate camera control unit. Image enhancement was achieved with an IVS Image I processor. Cells were recorded in real time on a VCR during all stages of feeding. Videos were then played back, individual images frozen and photographed off the monitor screen with a Pentax K 1000 camera (50 mm lens) using Kodak technical pan film 2415.

Results

Cells of *E. pulchra* are sessile, biflagellate unicells epiphytic on submerged vegetation or attached to planktonic organisms in freshwater habitats. Individual cells are surrounded by a vase-shaped lorica and are easily viewed with the light microscope, being ca. 26–37 μm

long and 3.5–6.8 μm wide. A single, elongate cell occupies each lorica and is attached to the base of the lorica by a basal stalk (Fig. 1 a–o) (also see Wetherbee et al. 1988). The anterior of the cell, with two flagella of unequal length, projects out through the lorica opening during feeding. The long flagellum is characterized by the presence of two rows of hairs (or mastigonemes) on its surface, while the short flagellum lacks obvious appendages.

Feeding appears possible throughout the cell cycle, except during stages of flagellar duplication and transformation, and when one daughter cell is briefly motile following cell division (Wetherbee et al. 1988). In motile as well as nonfeeding cells, the chloroplast and eyespot are positioned next to the flagellar bases, with the flagellar swelling of the short flagellum positioned opposite the eyespot, a situation characteristic of most chrysophycean algae (for review, see Andersen 1989). When feeding, cells become more elongate and the flagellar bases become clearly separated from eyespot and chloroplast (Fig. 1) (also see Andersen and Wetherbee 1991). The long flagellum is ca. 15–25 μm in length and displays a classic sigmoid beat when generating the water current that moves food particles toward the cell. The short flagellum is about one third the length of the long flagellum and remains relatively still during prey gathering (Fig. 1 a and b), though we have seen this flagellum beat in a similar fashion to the long flagellum under certain, unknown conditions.

The beat of the long flagellum creates a strong current that brings food particles toward the cell surface. Prey are normally delivered to a position between the two flagella that corresponds to the upper third of the small flagellum (Figs. 1 c–e, 2 b, and 4 b). On contact, the long flagellum instantaneously stops beating and reorients dramatically to seize the prey between itself and the short flagellum (Figs. 1 c–k, 2 a–c, 3 a–d, and 4 a and b). Relative to the long flagellum, the configuration of the short flagellum only alters slightly (Fig. 3 b), if at all (Fig. 1 d–h), during capture, though in a few instances the short flagellum was observed to bend back sharply to encircle prey.

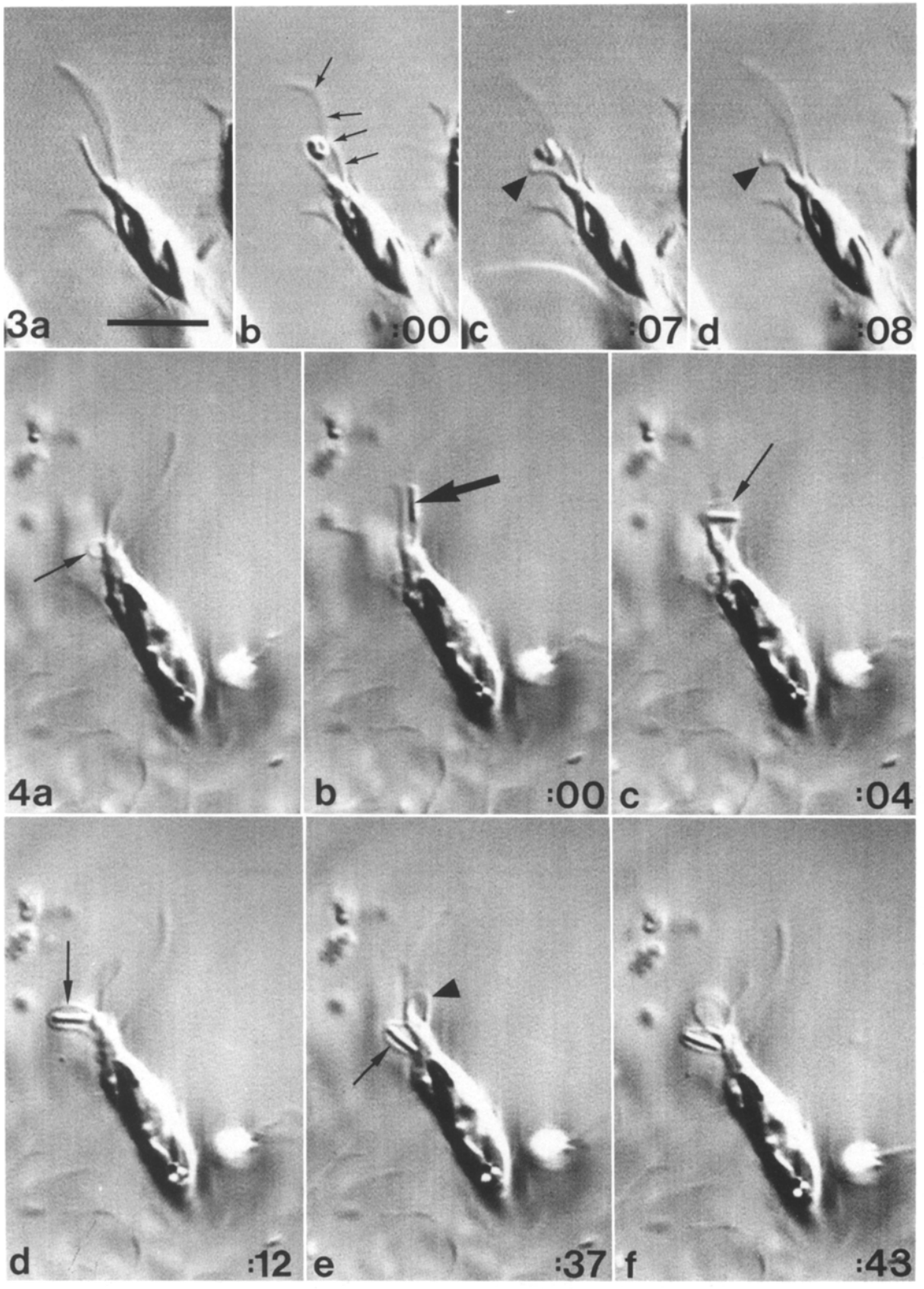
Particularly small prey are often trapped between the bases of the two flagella just above the cell surface, with no major contortion required by either flagellum (not illustrated). Overall, the manner of seizure seems to depend somewhat on the size (shape?, mass?) of the prey and the location of contact, with both flagella always contorting in such a way as to maximize entrapment.

Flagella appear to seize, at least briefly, all particles of a reasonable size that come in contact with them, though not all are selected. The prey are slightly rotated between the flagella while a decision on their desirability appears to be made (Figs. 1–4). Normally this takes 0.5–2 s, although we have observed cells to hold and rotate prey for up to 7 seconds (Fig. 3 b). If the prey is recognized as non-desirable, it is released by the flagella and quickly transported by water currents from the vicinity (Figs. 2 a–c and 3 c and d). Simultaneously the short flagellum bends sharply away from the seized prey, releasing it, while the long flagellum resumes its normal beat. The action of the small flagellum during prey release is consistent, with a single bend occurring approximately half way up its length, resulting in the flagellum bending back on itself at an angle of 90° or more (Figs. 2 c and 3 c).

If selected as desirable, the flagella hold the prey in place until a complex feeding cup, which appears as a loop-shaped profile under the light microscope, extends up from the apex of the cell near the basal bodies to engulf the victim (Figs. 1 f–o, 2 d–f, and 4). The cup starts forming almost immediately, within 1–2 s of capture, and envelopes the prey (Figs. 1 and 4) within ca. 2–4 s depending on the distance between the cell apex and the site of flagellar capture. The feeding cup with internalised particle, now appropriately termed a food vacuole, is then retracted more slowly back to the cell proper, taking ca. 5–15 s depending on the size of the prey and the proximity of the capture site to the cell apex (Figs. 1, 2 d–f, and 4). The food vacuole remains clearly visible near the cell apex for several minutes during digestion, and the contents are seen to noticeably breakdown during this time. Eventually the cell

Fig. 3. Frozen images of a video sequence (numerals refer to time in seconds) showing prey seizure and subsequent rejection. Note the contortion of both the long (small arrows) and short flagella during seizure (**b**). Prey is rotated for 7 s prior to release, the small flagellum (arrowhead, **c** and **d**) bending sharply away while the long flagellum resumes its normal feeding beat (**d**). Bar: 15 μm

Fig. 4. Frozen images of a video sequence (in seconds) showing that only one feeding cup can form at one time (**c** and **e**) while several food vacuoles can be retracted at any one time (**a** and **d–e**). An apparently empty food vacuole is being retracted (arrow, **a**) when prey is seized (arrow, **b**) between the two flagella and captured by a feeding cup (arrow, **c**) that becomes a food vacuole (arrow, **d**). An additional cup is formed (arrowhead, **e**) but is unsuccessful in capturing prey and is later retracted as a food vacuole (**f**) in the normal way. Bar: 15 μm


3a
b :00
c :07
d :08
4a
b :00
c :04
d :12
e :37
f :43

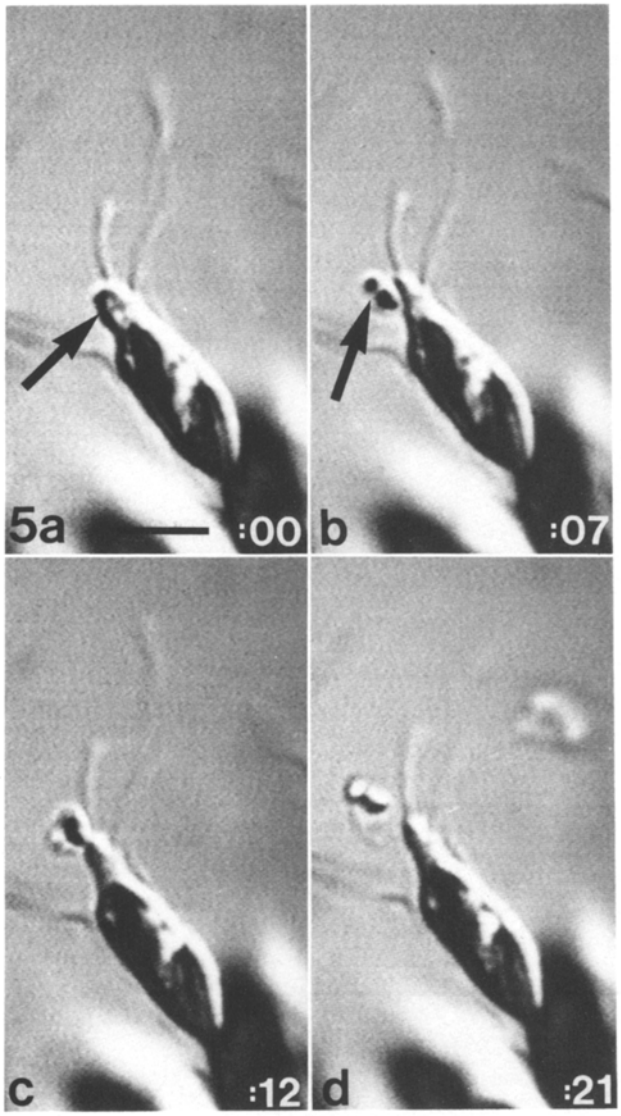


Fig. 5. Remains of undigested prey (large arrow) are expelled from a cell near its apex in a process that appears the reverse of prey capture (**a–d**). Remains appear within a halo prior to expulsion (**b–d**). Numerals on bottom right refer to time sequence in seconds. Bar: 8 µm

expels the undigested material, moving the remains the short distance to the cell apex where it is released and removed quickly from the area by the beat of the long flagellum (Fig. 5). The entire feeding event can take only 1–5 min.

Flagella release prey as the feeding cup engulfs it, quickly resuming their feeding orientation (Figs. 1 and 4). In some cases, particularly when small prey are captured between the bases of the flagella, two or three prey may be trapped in rapid succession and quickly transferred to a single cup (Fig. 2 d–f). Cells have been observed to create two or three feeding cups in relatively quick succession, particularly if they are unsuccessful at capturing prey, though we have only observed one cup growing out from the cell surface at any one time (Fig. 4). Only when a cup is transformed into a food vacuole (i.e., the cup opening is closed as discussed in Andersen and Wetherbee 1991) can a second cup form. On the other hand, cells can retract several food vacuoles (i.e., former cups) at any one time (Fig. 4), retraction proceeding much more slowly than cup formation.

Prey occasionally escape, either from the grip of the flagella or during transfer to the feeding cup. Cells may also reject prey previously selected by the flagella at this stage. This occurs when a cup comes into contact with prey but elects not to engulf it, or rejects it shortly after phagocytosis. There appears to be a number of stages where selection can occur.

Discussion

The nutritional versatility of another chrysophycean alga, *Ochromonas danica*, is well documented (Pringsheim 1952, Andersson et al. 1989, Sanders et al. 1990), and it now seems certain that *E. pulchra*, and possibly a wide range of organisms from the Division Chrysophyta, also act as mixotrophs (for review, see Sanders and Porter 1988). The implications are great, particularly for our understanding of productivity in aquatic ecosystems, and the interactions between mixotrophic flagellates and the populations of picoplankton they consume merit continuing investigation, including the cell biological aspects of phagotrophy.

Flagellar activity has held the curiosity of cell biologists since the advent of light microscopy. Since that time a significant degree of uniformity in flagella/cilia design has been established, particularly the organization of the highly conserved 9 + 2 central axoneme (for review, see Moestrup 1982). It is now generally accepted that the individuality of flagella, which is manifest in their diverse functions (e.g., beat pattern), is determined by the types of molecules (e.g., microtubule associated proteins) or structures associated with the basic flagellar organization. Although motility has been examined in detail, specialized flagellar functions, such as gliding and a role in gamete recognition during mating, have also received considerable attention (e.g., Bloodgood 1982, Goodenough and Adair 1989). These latter activities are attributable to specific organisms, often algae, and are frequently associated with distinct components located on the flagellar surface.

The mechanism of prey capture described here entails a level of flagella involvement not previously envisaged.

Although flagella have long been known to gather food particles to the cell surface with a characteristic beat (Cienkowshi 1870, Saville-Kent 1880, Pringsheim 1952, Aaronson 1973, Cole and Wynne 1974), in *E. pulchra* prey are physically seized between two flagella that orient themselves to best secure entrapment. The long flagellum undergoes a significant reorientation while the short flagellum normally contorts only slightly. As rows of stiff mastigonemes (or hairs) adorn the long flagellum, it would seem well equipped to aid in the seizure of prey by orienting in such a way as to best employ these structures. Following entrapment, flagella appear to have a recognition role, briefly rotating the prey prior to either selecting or rejecting it. Although we have not as yet done experiments that conclusively demonstrate a recognition role, our observations suggest that flagella are very discerning, selecting or rejecting prey of seemingly similar size, mass, etc. on the basis of some qualitative, unknown feature of the prey surface. A recognition function has been attributed to flagella before, notably during the mating process (e.g., Goodenough and Adair 1989), although flagella have not been implicated previously in prey selection. In *E. pulchra* recognition appears to occur at several different stages of capture, including the stage when the feeding cup first makes contact with the prey. The high degree of coordination observed between the two flagella of *E. pulchra* during prey seizure also occurs between the flagella and cell cytoplasm during the formation of the feeding cup. The prompt response of the feeding cup to prey capture strongly suggested to us that the flagellar root system, particularly the microtubular component, might be involved in the accurate formation of the cup and its precise orientation towards the prey prior to engulfing it. The structure of the flagellar root system in microalgae is a well known taxonomic and phylogenetic tool, and a role in prey capture could have important implications for how flagellar roots have evolved or even arose. These factors led to our investigations of the role of the flagellar apparatus in prey capture in *E. pulchra* which are discussed in the following paper (Andersen and Wetherbee 1992). Further investigations of prey capture in *E. pulchra* as well as other chrysophycean flagellates are underway, and include studies of cell–prey recognition and the molecules involved.

Acknowledgements

We thank Steven Platt and Lara Perasso for technical assistance and Jeremy Pickett-Heaps for reviewing the manuscript. RW acknowledges the support of the Australian Research Council and RAA the support of the National Science Foundation Grant no. BSR 8709222.

References

Aaronson S (1973) Particle aggregation and phagotrophy by *Ochromonas*. Arch Mikrobiol 92: 39–44

Andersen RA (1989) The Synurophyceae and their relationship to other golden algae. Beih Nova Hedwigia 95: 1–26

– Wetherbee R (1992) Microtubules of the flagellar apparatus are active during prey capture in the chrysophycean alga *Epipyxis pulchra*. Protoplasma 166: 8–20

Andersson A, Falk S, Samuelsson G, Hagstrom A (1989) Nutritional characteristics of a mixotrophic nanoflagellate, *Ochromonas* sp. Microbial Ecol 17: 251–262

Bloodgood RA (1982) Flagellum as a model system for studying dynamic cell-surface events. Cold Spring Harbor Symp Quant Biol 46: 683–693

Cienkowski L (1870) Über Palmellaceen und einige Flagellaten. Arch Mikrosk Anat Entwicklungsmech 6: 421–438

Cole GT, Wynne MJ (1974) Endocytosis of microcystic aeruginosa by *Ochromonas danica*. J Phycol 10: 397–410

Foster KW, Smyth RD (1980) Light antennas in phototactic algae. Microbiol Rev 44: 572–630

Goodenough UW, Adair WS (1989) Recognition proteins of *Chlamydomonas reinhardtii* (Chlorophyceae). In: Coleman AW, Goff LJ, Stein-Taylor JR (eds) Algae as experimental systems. AR Liss, New York, pp 171–186

James-Clark H (1867) On the Spongiae Ciliatae as Infusoria Flagellata; or, observations on the structure, animality, and relationship of *Leucosolenia botryoides*. Bowerbank Mem Boston Soc Nat Hist 1: 305–340

Moestrup Ø (1982) Flagellar structure in algae: a review, with new observations particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae and *Reckertia*. Phycologia 21: 427–528

Pringsheim EG (1952) On the nutrition of *Ochromonas*. Q J Microsc Sci 93: 71–96

Sanders RW, Porter KG (1988) Phagotropic phytoflagellates. In: Marshall KC (ed) Advances in microbial ecology. Plenum, New York, pp 167–192

– Porter KG, Caron DA (1990) Relationship between phytotrophy and phagotrophy in the mixotrophic chrysophyte *Poteriochromonas malhamensis*. Microbial Ecol 19: 98–109

Saville-Kent W (1880) A manual of Infusoria. 1. London

Stokes AC (1885) Notes on some apparently undescribed forms of fresh-water infusoria, no. 2. Am J Sci III 29: 313–328

Wetherbee R, Platt SJ, Beech PL, Pickett-Heaps JD (1988) Flagellar transformation in the heterokont *Epipyxis pulchra* (Chrysophyceae): direct observations using image enhanced light microscopy. Protoplasma 145: 47–54