A new kind of motility mutant (non-gliding) in *Chlamydomonas*

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Summary. Certain mutants of *Chlamydomonas moewusii*, induced by irradiation with UV-light, lack the flagellar gliding motility normally found in wild-type cells.

Most green algal flagellate cells can swim by frequent breast-stroke movements of their paired flagella. At 20–30 °C, beating at about 30 Hz, they swim at speeds of 100–200 μm sec^{-1}. A variety of induced mutants of *Chlamydomonas moewusii* and *C. reinhardtii* have been studied in which, as a consequence of microtubule deficiencies or other impairments of the axoneme, the cells are paralyzed and cannot swim[2–4]. Such cells can still move, however, by a sort of gliding, whereby flagella in contact with solid substrates (e.g., the glass of a microscope slide) draw the cells along the surface at about 2 μm sec^{-1} (fig., 1 and 2). This gliding ability occurs in wild-type cells, too[5], but because of their more rapid motion it is usually less apparent. It is most readily recognized by the position which many cells adopt when attached to a surface, e.g., of a glass slide: they become 'spread-eagled' as the flagella, pulling in opposite directions, bring the cell body into contact with the substratum (fig., 3 and 4). I have now obtained double mutants which are impaired not only in their swimming but also in their gliding ability. They should permit genetic studies of flagellar surface movements unconnected, apparently, to those of the axial microtubules.

I used a simple technique for selecting motility mutants, which had proved successful for *Euglena* in earlier studies[8]. Cells of a *C. moewusii* mutant, M.475, with paralyzed flagella (pf^-)[3] were irradiated at 10 cm from a mutagenic UV-light source ('sterilamp') for 45 sec; incubated overnight in darkness to preclude photoreversal; grown for 3 days in continuous light at 21 °C to permit about 5 doubling generations; and plated in 0.3% agar medium[3]. After further illumination for 10 days, colonies of different sizes could be distinguished. The majority were large, about 3–4 mm in diameter; they comprised cells of the original M.475 type which, despite flagellar paralysis, had been able to glide outwards for a short distance through the semisolid medium. (Under similar circumstances, wild-type cells capable of normal swimming form colonies up to 10 mm in diameter.) The smaller colonies comprised cells which were further impaired in motility or cell division: among 100 colonies picked and transferred to liquid medium, most were palmelloid or flagella-less, but 6 (possibly all originating from a single mutant) were unable to glide.

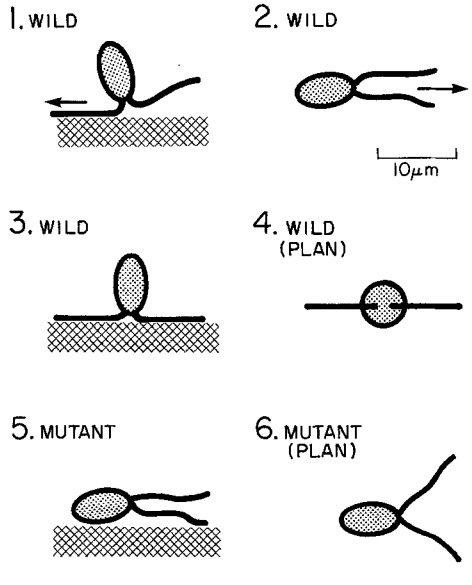
These cells were of normal appearance and had flagella of normal length (12–15 μm), but, unlike the parent strain, they did not slither along surfaces. The flagella twitched a little distally, like those of the parent strain, but they stuck to a substratum only by their tips or not at all (fig., 5). In microscope mounts of these mutants the living vegetative cells did not adopt the spread-eagled flagellar configuration of wild-type or merely paralyzed cells, with the paired flagella 180 °C apart; instead, they usually came to lie on their sides with the flagella angled forwards (fig., 6). I designate such strains as (flagella) non-gliding (fg^-) mutants.

Electron-micrographs of flagellate cells, negatively stained with uranyl acetate or phosphotungstate, showed no differences between fg^- and fg^+ flagellar surfaces: both appeared smooth, with no sign of lateral hairs.

Gliding motility is generally revealed not only by translocation of cells over substrates but also by translocation of particles along flagellar surfaces. Microcinematographic studies by Bloodgood[6] elegantly demonstrated the latter phenomenon: polystyrene beads, adherent to flagella of *Chlamydomonas reinhardtii* or other species, were moved to and fro at speeds of 1.6–2.2 μm sec^{-1}. I used a simple modification of his technique to compare the flagella of fg^- mutants with those of fg^+ cells (pf^- in both cases, to facilitate observations).

Polystyrene beads 0.45 μm in diameter (Dow Chem. Co., Indianapolis, Ind., USA) were mixed with *Chlamydomonas* cells suspended in distilled water. (The paucity of cations in the medium was presumably conducive to the adhesion of the beads to the flagellar surfaces, which are electronegatively charged: no anti-flagella antibodies were needed.) Beads attached to fg^+ flagella at various positions, and by phase-constrast microscopy they could be readily seen to slide to and fro. Particles moving proximally tended to accumulate in the region of the flagellar base, from which they often detached. Particles moving distally, if they reached the flagellar tip, rarely detached; usually they moved back again towards the cell body. Distad movement, though observed and reported by Bloodgood, was not expected, since on solid substrates flagellar gliding is always tip-first. Beads attached less readily to fg^- flagella except at the tips, where electron-micrographs revealed a strand of something that might be 'sticky' and thus responsible for tip attachment (fig., 5). On fg^- flagella, attached polystyrene beads were not moved to and fro.

One may speculate on the role of flagellar gliding. It may enable cells which accidentally adhere to solid bodies such as soil particles to move across or around the obstacles. It may enable them to move in or out of crevices too narrow to permit normal swimming movements. And it may also serve to disengage small particles of dirt that become attached to the flagella, by moving them proximad towards the flagellar bases where they tend to fall off. At present we



do not know what molecular mechanisms might underlie this kind of movement. It may be driven by a system similar to that responsible for gliding in flexibacteria[7] and many filamentous blue-green algae[9] but, for these movements, too, there is still no satisfactory explanation. Using a somewhat more tedious isolation technique, Glaser and Pate[10] have obtained non-gliding mutants of a flexibacterium, *Cytophaga columnaris*. Comparative studies of such organisms may ultimately help us to elucidate cellular mechanisms underlying gliding motility.

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Reduction in bean common mosaic virus (BCMV) infectivity vis-a-vis crude leaf extract of some higher plants

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Summary. Crude leaf extracts of 17 plants were tested for their antiviral activity against BCMV. An extract of *Azadirachta indica* A. Juss. was found to be most potent in reducing the infectivity of the virus.

Several workers have reported inhibitory activity of juices of several plants including Strawberry[2], *Phytolacca esculenta*[3], Tobacco leaves[4], *Achyranthes aspera, Aloe barbadens, Capsicum frutescens* and *Carica papaya*[5] and various medicinal plants[6]. The present investigation is concerned with the antiviral activity of the crude leaf extracts of some higher plants.

Legumes are the best source of protein. During the survey of leguminous plants of Eastern U.P. (India), bean common mosaic virus (BCMV) was found to be most noxious. It was found to be perpetuated in cultivated plants viz. *Vigna sinensis* Roxb., *Phaseolus vulgaris* L., and certain other pulses including weeds like *Crotolaria striata* L. Due to the prevalent nature of the virus it was considered desirable to search for antiviral agents.

Bean common mosaic virus: effect of crude leaf extracts on infectivity (test plant *Chenopodium amaranticolor)*

Plant species	Average No. of lesions	Reduced infectivity %
Clerodendrum inerme (L.) Gaertn.	20±0.05	0
Xanthium strumarium Linn.	20±0.08	0
Putranjiva roxburghii Wall.	20±0.09	0
Erigeron molle. D. Don.	18±0.15	10
Eclipta alba Hassk.	14±1.20	30
Pongamia pinnata (L.) Pierrc	20±0.04	0
Eucalyptus lanceolatus L. Herit.	16±0.88	20
Leucas aspera (Willd.) Spreng.	18±0.67	10
Leucas procumbens Desf.	17±0.19	15
Sapindus emarginatus Vahl.	20±0.05	0
Santalum album Linn.	20±0.12	0
Azadirachta indica A. Juss.	1±0.89	95
Croton bonplandianum Baill.	17±1.50	15
Mangifera indica Linn.	20±0.10	0
Ocimum sanctum Linn.	10±0.24	50
Lantana indica Roxb.	18±0.95	10
Raphanus sativus L.	17±0.09	15
Distilled water (Control)	20±0.00	0

±SEM.

Cultures of BCMV were maintained in systemically infected *Vigna sinensis* L., in an insect-proof glass house. The inoculum was prepared by grinding 1 g of young diseased leaves in a mortar, and the juice was expressed by squeezing the pulp through muslin cloth, diluted 1:10 with distilled water and kept at 20 °C. The plant extracts were made by the same method, using leaves of different plants. The inhibitory effect of the crude leaf extracts was determined by comparing the infectivity of equal volumes of BCMV and distilled water (control) with equal volumes of BCMV and extract by the local lesion method. The mixtures were rubbed after 10 min of mixing on *Chenopodium amaranticolor* Coste et Reyn[7] (local lesion plant) leaves. 5 replicates were taken for each treatment. Each mixture was inoculated on 20 half-leaves and the half-leaves alloted to each treatment were distributed among plants so as to form a randomized block. Carborundum powder was dusted on leaves before inoculation and inoculation were made with a forefinger wet with inoculum. The results, showing reduced infectivity of BCMV after treatment with crude leaf extracts, are summarized in the table.

The results shown indicate that the infectivity of BCMV was reduced by 10 to 95% in different crude saps of plants. Maximum reduction in infection was observed with *Azadirachta indica* A. Juss. and minimum with *Erigeron molle* D. Don., *Leucas aspera* (Willd.) Spreng and *Lantana indica* Roxb.

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