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Orientation in two dimensions: chemosensory motile behaviour of *Euplotes vannus*

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Abstract

Most studies on chemosensory motile behaviour of protists apply to free-swimming species moving in 3-dimensional space. But many protists are associated with surfaces and this excludes the use of helical klinotaxis for orientation in chemical gradients. It is here shown that the predominantly surface-dwelling ciliated protozoon *Euplotes vannus* orients itself in chemical gradients by simple temporal gradient sensing (equivalent to the run and tumble mechanism described for bacteria) and they react only to a temporal decrease in attractant concentration. The motility of *Euplotes* can be described as a classical 2-dimensional random walk with Poisson distribution of run lengths punctuated by random changes in walking direction. The chemosensory motile behaviour allows cells that are distributed within about 1 cm^2 to accumulate at point sources of an attractant within 3–4 min.

Keywords: *Euplotes vannus*; Chemosensory motile behaviour; Temporal gradient sensing; Chemotaxis; Lévy walks

Introduction

It is generally assumed that microorganisms cannot directly determine the direction of a chemical gradient. Instead they apply so-called temporal gradient sensing. While moving through a chemical gradient cells sense a temporal change in concentration to which they respond by changing swimming direction. In the classical case of bacterial chemosensory behaviour changes in swimming direction are random, but cells change swimming direction more frequently when they sense a decreasing concentration of an attractant and less frequently when they sense an increasing concentration. This leads to a biased random walk towards the source of an attractant, but the swimming direction is at any time random relative to the gradient.

Recent studies have shown that the so-called helical klinotaxis is a predominant mechanism for orientation in chemical gradients among free-swimming protists (Blackburn and Fenchel, 1999; Fenchel and Blackburn, 1999; Fenchel, 2001) and even for a very large bacterium (Thar and Fenchel, 2001). This mechanism, originally suggested and analysed by Crenshaw (1993a, b), implies that cells utilise their inherent tendency to swim in a helical path. When the axis of the helical swimming path is crossing a concentration gradient of an attractant, cells sense a change in concentration during a half cycle of the helix. This again leads to changes in the axial rotation velocity of the cell so that the axis of the helical swimming path bends towards higher concentrations. The swimming direction is thus oriented relative to the concentration gradient and the cells approach a point source directly with a velocity that is close to the actual swimming speed. But many protists always or predominantly move along surfaces and helical klinotaxis would seem to be precluded in this case. Here the mechanism employed by a hypotrich ciliate *Euplotes vannus* to move towards a point source of an attractant has been studied.

There have been previous studies of motility of *Euplotes*. Ricci et al. (1987) provided a detailed

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description of an apparently very complex motile behaviour of *E. crassus*, but only limited insight into the adaptive significance of the motility patterns. Jonsson and Johansson (1997) found that accumulation of a *Euplotes* sp. in food patches was due to avoidance reactions when leaving the patches. They also found that starvation induces swimming and emphasised its role for long-range dispersal through advective transport.

It is usually assumed that in a homogeneous environment the random walk of microbes shows a Poisson distribution, that is, that the probability of changing direction is constant, as in Brownian motion, although this has rarely been critically tested (Berg, 1983; Blackburn and Fenchel, 1999). However an alternative type of random walk, the so-called Lévy walk, has recently drawn interest. In Lévy walks the probability distribution of longer time intervals (or path lengths) decreases as a power function in contrast to the exponential decay of the Poisson distribution. This results in an excess of very long straight swimming or walking paths. It can be shown theoretically that this enhances searching relative to a classical random walk, because it covers a larger area during a given period of time and because crossing back trails is less frequent (e.g., Viswanathan et al., 1999). Lévy walks have been found in e.g., foraging bumblebees and albatrosses (Heinrich, 1979; Viswanathan et al., 1996). They have also been implied for plankton protists (Levandowsky et al., 1988) and demonstrated for amoebae (Levandowsky et al., 1997; Schuster and Levandowsky, 1996). A second purpose of the present study was to accurately describe the motility pattern of a food-seeking ciliate.

Material and methods

E. vannus was originally isolated from Nivå Bay about 25 km north of Copenhagen. Stock cultures were maintained as wheat grain cultures in 20 ppt seawater at 10°C. For experiments, 200 ml batch cultures were prepared with a suspension of an unidentified marine bacterial isolate (initially about 10^8 cells ml^{-1}) in otherwise particle-free seawater. The cultures were kept at room temperature (about 22°C). Cells for experiments were harvested during late exponential growth or within 24 h after growth had stopped. Cells were washed and concentrated on a sieve with nylon net (mesh size: 15 μm) and re-suspended in particle free seawater (''starving cells''). Alternatively, a fresh bacterial suspension was added (''feeding cells'').

Cell motility and accumulation at attractant sources were recorded in plastic petri dishes or in capillary microslides (Camlab, Cambridge) with an internal width of 4 mm and a height of 0.4 mm. A dissection microscope with 6 or 12 × magnification and dark field illumination based on a fibre-optical lamp with heat filter was fitted with a CCD-camera connected to a digital video recorder. Cell numbers and positions were later recorded from the monitor. Swimming tracks of individual cells were analysed from the videotapes with a computer and a software program LabTrack (DiMedia, Kvistgaard, Denmark) using different time steps (mainly 0.16 or 0.32 s).

Bacteria embedded in 2% seawater agar were used as attractant (about 5 μl bacteria scraped from a nutrient agar plate mixed into about 2 ml warm agar). Embedding the bacteria in agar prohibited the ciliates from feeding so that the protozoa could only respond to the concentration gradient of dissolved organic matter diffusing from the agar. A liquid drop of the bacteria-agar (diameter 0.5 mm) was applied to the centre of a plastic petri dish. After the agar had congealed, a suspension of *Euplotes* was added and an area of 10×7.2 mm or of 5×3.6 mm surrounding the agar patch was monitored for up to 10 min (radial concentration gradient, point source). Alternatively, a capillary microslide was filled with a *Euplotes* suspension and one end of the slide was pressed into solid bacteria-agar leaving a 1–2 mm agar plug in one end and the accumulation of ciliates in front of the agar was followed (linear concentration gradient).

Measurements of dispersal rates were based on records of walking tracks (exemplified in Fig. 1). The distance from the starting point of a track covered by a ciliate was measured at 3.2 s intervals. Altogether 40 tracks of starving and 40 tracks of feeding ciliates were analysed and standard deviations of the mean displacements were calculated.

Results

Motile behaviour in a homogeneous environment

Euplotes cells can swim, but the cells practically only swam following mechanical disturbance (stirring or shaking cultures); otherwise the vast majority—even of starving cells—walked on the bottom of containers. Examples of walking tracks in a homogeneous environment of starving and of feeding *Euplotes* are shown in Fig. 1. The walking speed of starving cells was 0.43 mm s^{-1} (SD: 0.07 mm s^{-1}, $n = 20$) and that of feeding cells was 0.22 mm s^{-1} (SD: 0.05 mm s^{-1}, $n = 30$). The difference between the two groups is, however, somewhat larger because feeding cells tend to stop at intervals of a few seconds while feeding and these stops were not included when calculating velocities. Also feeding cells change walking direction more frequently and this effectively decreases the average walking velocity because each change in direction means that the ciliate effectively stops moving for 0.6–0.9 s.

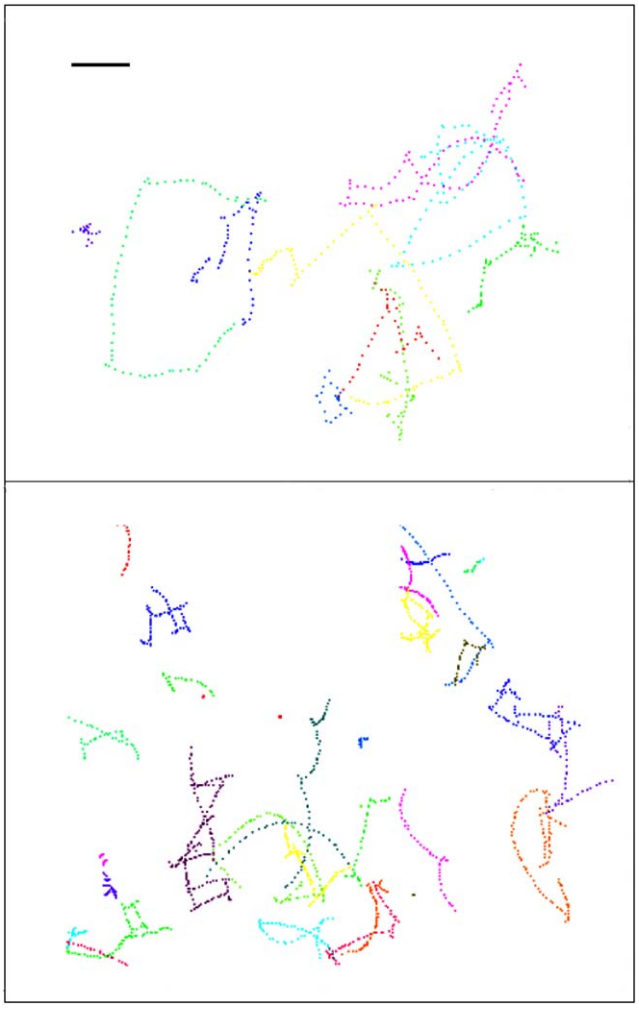

Fig. 1. Tracks of walking *E. vannus* in homogeneous environments. Above: cells in particle free seawater; below: with a bacterial suspension. Scale bar: 1 mm; time resolution (distance between dots): 0.32 s.

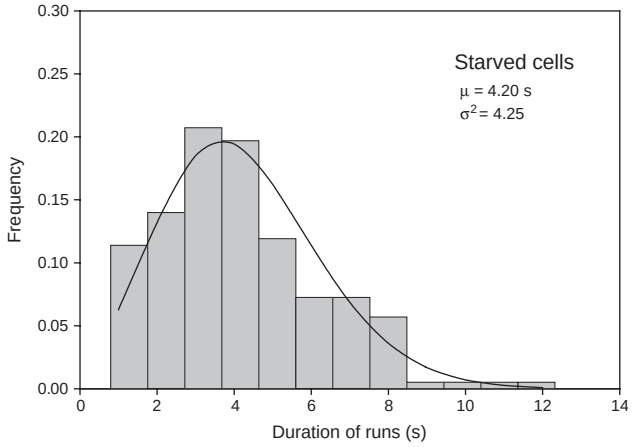

Fig. 2. Frequency distribution ($n = 193$) of time between successive changes in walking direction of starving cells and data fitted to a Poisson distribution with a mean of 4.2 s.

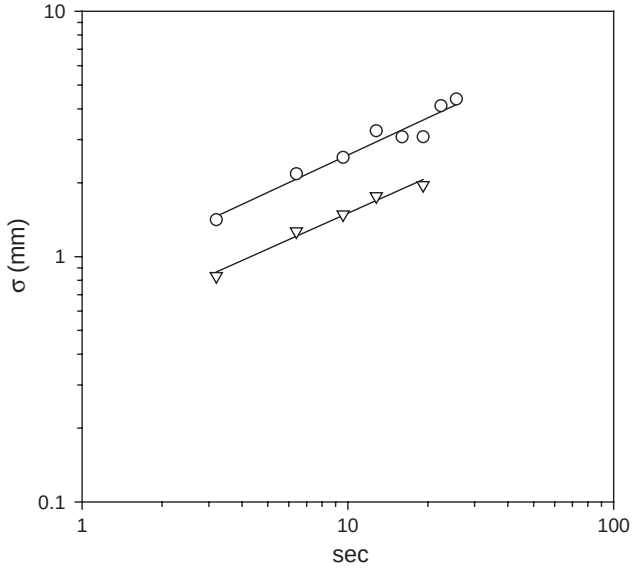

Fig. 3. Standard deviations of displacement of *Euplotes* cells as function of time. Circles: starving cells; triangles: feeding cells. The slopes of the regression lines are 0.504 ($r^2 = 0.95$) and 0.484 ($r^2 = 0.98$) mm s^{-1}, respectively. Extrapolating the lines to 1 s, squaring the result, and dividing by 4 (to obtain 1-dimensional "diffusion") is a measure of motility (Berg, 1983). Each curve is based on 40 tracks.

Walking paths consist of linear or slightly curved segments (runs) punctuated by abrupt changes in walking direction (Fig. 1). The time interval distribution is consistent with a Poisson distribution (constant probability of changing walking direction) with a mean of 4.20 s and a variance of 4.25 for starving cells (Fig. 2). Feeding cells changed walking direction more frequently (mean interval: 2.80 s). In this case the fit to a Poisson distribution was poorer (variance: 1.96), but this is probably an artefact because the time resolution of the tracks was 0.32 s and so the frequency of short (<1 s) intervals of turning was probably underestimated.

The dispersal of cells yielded a Gaussian distribution and the standard deviation increased in proportion to the square root of time (Fig. 3). In analogy to molecular diffusion, a "diffusion coefficient" (motility) can be expressed as the increase in variance per unit time. Motilities for starving and feeding cells were 0.14 and 0.04 $mm^2 s^{-1}$, respectively.

The turning angles were almost equally distributed between 45° and 315° while smaller deviations between the previous and following walking direction were rare, constituting <5% of all turns ($n = 63$). It is possible, however, that this result is biased because changes in walking direction could have been overlooked when

deviations between the previous and the following walking directions were slight.

Behaviour in an attractant gradient

Euplotes cells rapidly accumulated in the vicinity of agar with embedded bacteria (Fig. 4) and the cell concentration close to the agar (<0.5 mm) was about 100 times higher than the mean concentration in the dishes after 3–4 min. Initially the rate of accumulation was almost linear and then gradually decreased as an increasing area surrounding the attractant source was depleted of ciliates thus approaching a steady-state distribution. Later, after 8–10 min, the cell density adjacent to the agar tended to decrease again; most likely this was caused by a dilution of attractant molecules diffusing out from the agar. At the time of maximum density of ciliates close to the agar, their density declined exponentially with distance from the surface of the bacteria-agar (Fig. 4, below). In experiments with a point source (radial concentration gradients) all the ciliates within an area of about 1 cm^2 had accumulated close to the surface of the agar after about 3 min.

Tracks recorded in the vicinity of an attractant shortly after the start of an experiment do not immediately reveal how the ciliates manage to accumulate (Fig. 5). One way to analyse the tracks was to measure the angle ϕ between vectorised tracks and a line perpendicular to the (assumed) chemical gradient. Angles $0<\phi<\pi$ mean that tracks are directed towards the attractant source and angles $\pi<\phi<2\pi$ mean that tracks are directed away from the source (Fig. 6). The sine of the angles then quantifies the directions of tracks towards (positive values) or away from (negative values) the source. The mean sine value of ϕ proved positive to a distance of about 3 mm from the agar surface. Further away it approached 0 that is the expected value if the directions of the tracks are random. Thus the ciliates responded to the gradient to a distance of about 3 mm from the agar surface. Looking separately at tracks with $\sin\phi > 0$ and with $\sin\phi<0$, then expected mean values should be $\sin\pi/4$ (=0.707) and $\sin 5\pi/4$ (=−0.707), respectively, if they have random directions. This appeared to be the case at all distances from the attractant (Fig. 6). This implies that the walking direction is not at any distance oriented relative to the chemical gradient, but simply that in the vicinity of the attractant source, tracks moving towards it tend to be longer than those moving in the opposite direction.

Fig. 7 shows how this is accomplished. Within a distance of about 3 mm from the attractant source, tracks pointing away from the source change direction

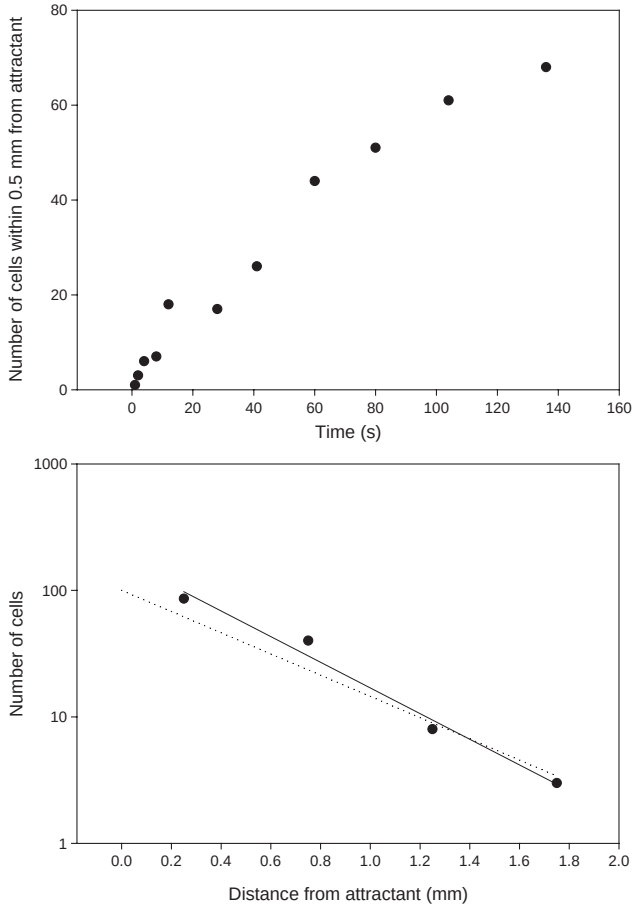

Fig. 4. Above: the accumulation of *Euplotes* cells in front of a bacteria-agar plug in a capillary microslide. The initial increase in cells is consistent with the model prediction of 1.3 cells s^{-1}. Below: same experiment as above showing the exponential decrease in cell density as function of distance after 3 min. Also shown (dotted line) is the model prediction.

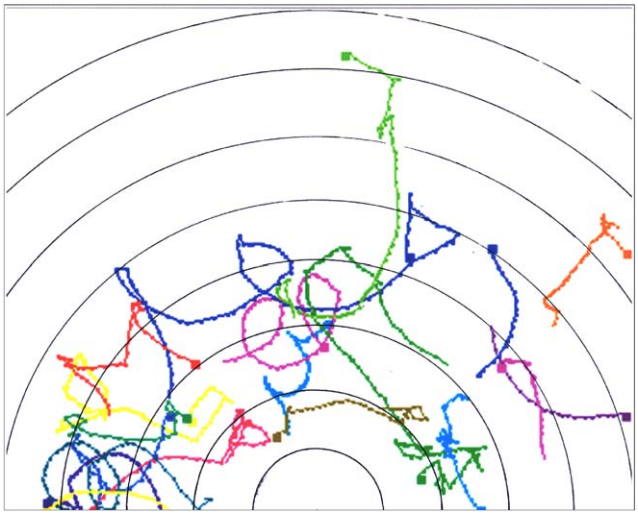

Fig. 5. Point source attraction of *Euplotes* cells (showing only half of the field) about 1.5 min after incubation. Start of individual tracks are marked by a square. Distance between half-circles is 0.5 mm and the innermost one contained a drop of bacteria-agar. Time intervals between dots: 0.32 s.

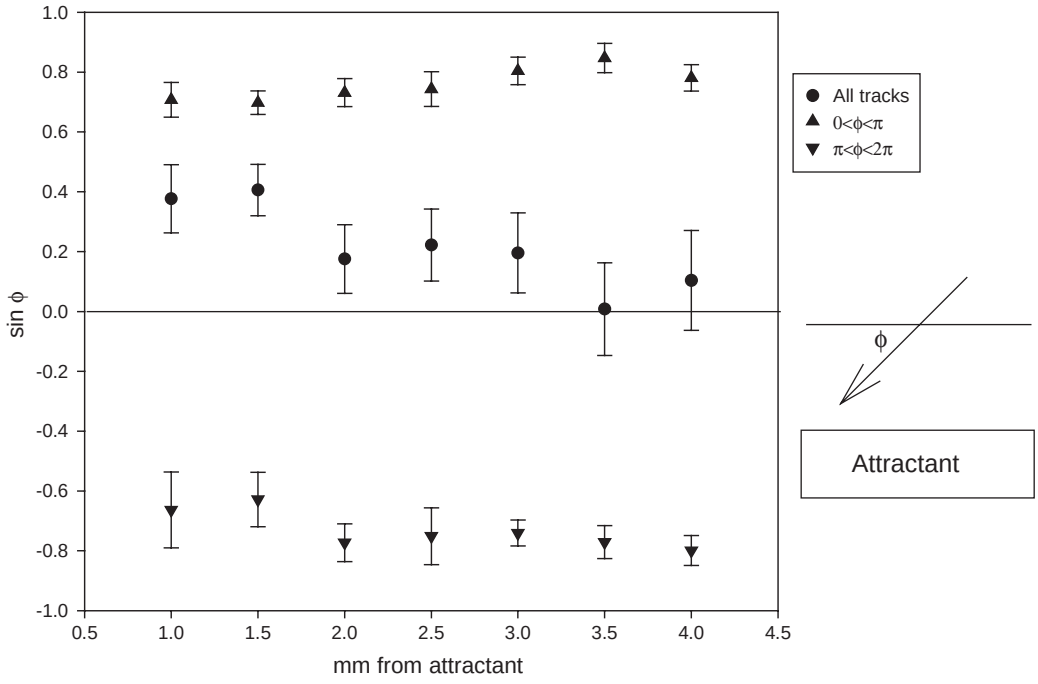

Fig. 6. Sine values of track angles as function of distance from attractant. The data set derives from the first 3 min of a point source experiment and represents 125 measured angles; bars represent one standard error.

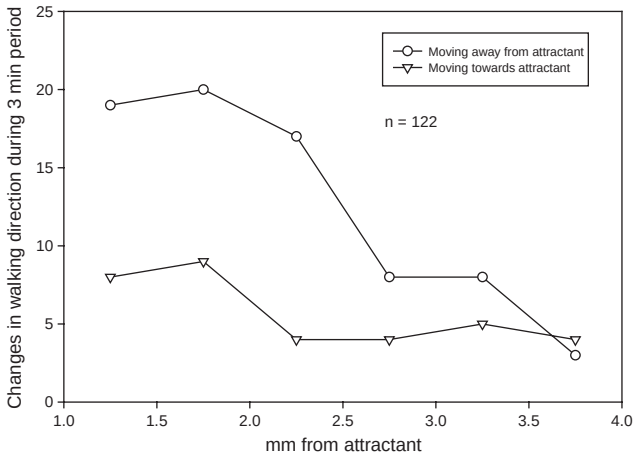

Fig. 7. Number of changes in walking direction in tracks leading towards and away from attractant during the first 3 min of a point source experiment.

2–3 times more frequently than tracks pointing towards the source. Changes in direction were nearly random and so a change in walking direction does not necessarily imply that the new direction is towards the attractant source, but the mechanism will still lead to a drift of the population towards the source. It should also be noted that the ciliates seem only to respond to a decrease in attractant concentration (moving away) whereas turning frequency seemed unaffected when the ciliates approach the attractant source. The walking velocity of the cells was constant irrespective of the walking direction relative to the attractant.

Modelling the accumulation of cells

The accumulation of the cells towards the attractant is a biased random walk so that the flux of cells has a drift term and a diffusion term: $J = \mathbf{v}n - D\,\partial n/\partial x$ (see also Lapidus and Levandowsky, 1981). Here n is areal density of the cells, x is distance, D is motility, and $\mathbf{v}$ is drift. The bias in walking direction is given as $1/n\sum\sin\phi$ (Fig. 6) and the drift $\mathbf{v}$ is then $v(1/n\sum\sin\phi)$ where v is walking velocity. Initially, when ciliates are randomly distributed ($\partial n/\partial x = 0$), $J = \mathbf{v}n$. At steady state when $J = 0$ we have that $\partial n/\partial x = \mathbf{v}n/D$ or, solving for n, $n(x) = n(0) \times \exp(-x\mathbf{v}/D)$ so that the concentration of cells decreases exponentially with distance from the attractant. From Fig. 6, $1/n\sum\sin\phi \approx 0.4$ (within about 2 mm from the agar surface) and for non-feeding *Euplotes*, $v = 0.43\,\mathrm{mm\,s^{-1}}$ and $D = 0.14\,\mathrm{mm^2\,s^{-1}}$.

The model predicted the initial flux of ciliates to the inner 0.5 mm zone adjacent to the bacteria-agar well in all cases. In the example shown in Fig. 4, the mean areal density of *Euplotes* cells $n = 2.1\,\mathrm{mm^{-2}}$ so the initial flux should be $2.1 \times 0.4 \times 0.43 = 0.36\,\mathrm{ciliates\,mm^{-1}\,s^{-1}}$. The length of the recorded 0.5 mm wide area adjacent to the agar was 3.6 mm so the initial flux of ciliates should be $3.6 \times 0.36 = 1.3\,\mathrm{ciliates\,s^{-1}}$. This is close to the observed initial flux. The concentration gradient of cells (Fig. 4, below) after 3 min was, however, somewhat steeper than predicted from the model, and this was the case in all experiments.

Discussion

The results show that the chemosensory motile behaviour in surface-dwelling *Euplotes* depends on an increase in frequency of random re-orientation of the walking path when cells experience a decrease in concentration of an attractant. The mechanism is therefore basically a 2-dimensional version of the chemosensory motile behaviour described for bacteria such as *Escherichia coli* (Berg, 1983). The velocity of the approach towards attractants is therefore only a fraction of the walking velocity. This is in contrast to swimming protists that through helical klinotaxis can move almost directly towards attractants with a velocity that approaches swimming velocity (Blackburn and Fenchel, 1999). Moving on a 2-dimensional surface precludes exploitation of the precision of a helical swimming trajectory. When the walking cells make a turn in a new direction they have no cue with respect to the direction of the gradient, and turning angles are therefore random. Even so, *Euplotes* can rapidly find point sources of food within a cm scale.

The present observations confirm those of Jonsson and Johansson (1997) that *Euplotes* cells perform avoidance reactions when leaving a patch of food and that this explains accumulation in food patches. In the present study chemosensory attraction was studied independently of access to food particles, showing there is a direct response to concentration gradients of dissolved attractants. The chemical nature of these attractants is unknown, but it has previously been shown that many bacterivorous protists respond to low molecular weight organic compounds such as amino acids as well as to more complex organic molecules (e.g., Hellung-Larsen et al., 1986; Fenchel and Jonsson, 1988). When patches contain available food particles, *Euplotes* decreases its walking speed by almost a factor of 2, a fact that was also noted by Jonsson and Johansson. This will enhance accumulation in food patches because, everything else being equal, the probability of staying in a patch is inversely proportional to the local velocity (Schnitzer et al., 1990). In the present study this did not apply because the ciliates were not exposed to available food particles. It is known that some hypotrich ciliates show preference for certain surface textures (Ricci et al., 1989). It is therefore possible that the texture of the agar surface is preferred relative to plastic or glass and this may contribute to the accumulation of cells closer to the bacteria-agar than predicted from the biased random walk model and independently measured motility parameters (Fig. 4).

The effect of chemosensory motile behaviour is local in an environment with discrete food patches. In the present experimental design this means within about 3 mm away from the attractant source. This figure may not necessarily be realistic for all situations in nature, but must depend on the nature of the diffusing compounds, concentrations, and steepness of the gradients. But under all circumstances the effect of the chemosensory behaviour is basically to make target patches appear larger so that they are more likely to be found through random search. In the studied *Euplotes* species, this random search is a classical Brownian motion type random walk and it was not possible to confirm the presence of Lévy walks as it has been found for some amoebae (Levandowsky et al., 1997; Schuster and Levandowsky, 1996). In a classical random walk the mean square deviation grows in proportion to time as found in the present study; in Lévy walks the mean square deviation grows as a power function of time with a fractal exponent between 1 and 2 (Levandowsky et al., 1997). Jonsson and Johansson (1997) showed that for an unidentified *Euplotes* species up to 7% of starving cells were swimming at any time. Water currents above surfaces may further result in wider transport of swimming cells. Such a mechanism may also serve to cover a larger area and to avoid back tracking. The ciliate strain used in the present study, however, did not tend to swim when deprived of food.

Acknowledgements

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