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Swimming patterns of the quadriflagellate *Tetraflagellochloris mauritanica* (Chlorophyceae, Chlamydomonadales)[1]

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Abstract

Chlamydomonadales are elective subjects for investigation of the problems related to locomotion and transport in biological fluid dynamics, whose resolution could enhance searching efficiency and assist in the avoidance of dangerous environment. In this paper we will try to elucidate the swimming behavior of *Tetraflagellochloris mauritanica*, a unicellular-multicellular alga belonging to the order Chlamydomonadales. This quadriflagellate

alga has a complex swimming motion consisting of alternating swimming phases connected by in-place random reorientations and resting phases. It is capable of both forward and backward swimming, both being normal modes of swimming. The complex swimming behavior resembles the run-and-tumble motion of peritrichous bacteria, with in-place reorientation taking the place of tumbles. In the forward swimming *T. mauritanica* shows a very efficient flagellar beat, with undulatory retrograde waves that run along the flagella to their tip. In the backward swimming, the flagella show a non-stereotypical synchronization mode, with a pattern that does not fit any of the modes present in the other Chlamydomonadales so far investigated.

Introduction

Life in water strongly conditions the shape and size of phytoplankton. Shape and size in turn affect both metabolism and motility, since cells need to access nutrients, whose diffusion are limited by gradients, and have to harvest enough light to fulfill photosynthetic requirements, despite sinking (Guasto et al. 2012). Uptake and propulsion must be simultaneously optimized to explore and exploit environmental resources (Barsanti and Gualtieri 2014).

Green algae belonging to the order Chlamydomonadales are model organisms to investigate swimming hydrodynamics involved in exploration and exploitation of environmental resources (Goldstein 2014). Chlamydomonadales are all good swimmers and control their level in the water column mainly by this means. Swimming means that an organism immersed in a liquid environment is able to sustain movement by deforming its body shape (cell body plus flagella) in a cyclic way (Lauga and Powers 2009). The world of microorganisms is the world of low Reynolds number, where inertia plays little role and viscous forces dominate the flow (Purcell 1977). Moreover, the low R physics imposes constraints on the possible shape changes that will result in progress. Considering some cyclic motion of a low R microalga swimmer, all the possible shapes of the swimmer can be constructed. Then the motion of the swimmer deformable body undergoing an ordered sequence of body-shape changes will be described by rotation and displacement of the body to its appropriate orientation and location. Therefore, in absence of inertial effects, the sequence of shape changes completely specifies the rotation and displacement of a body (Guasto et al. 2012).

Propulsion strategies have evolved that successfully overcome and exploit viscous drag. How then is propulsion achieved at low R? In the case of Chlamydomonadales, we can assume that successful propulsion relying on repetitive cyclical motion is assigned to the rhythmic beating of flagella. The hydrodynamics adequacy of their stroke for locomotion is quantified by the swimming efficiency, which can be defined as the power consumption

to swim a certain distance compared with the power one would need to drag the body through the fluid (Ishimoto and Gaffney 2014). Since at low R numbers thrust is generated by the same mechanism that resist motion, i.e. viscous drag, swimming efficiency is only a few percent. Its value mainly depends on the flagellar length, the payload to transport (i.e. the cell body), the ratio between flagellar and cell body length, and the amplitude and wavelength of the flagellar undulatory wave. For the mathematical formalization refer to Guasto et al. (2012).

In the Chlamydomonadales, flagella are organized in pairs, and motile cells are typically biflagellate, such as *Chlamydomonas*, or *Dunaliella*. The beating of the flagella in a pair needs some sort of synchronization to generate an efficient swimming strategy. Swimming cells can switch between synchrony and asynchrony, and flagella within a single organism can be functionally distinct (Pikovsky et al. 2003). Oscillations of the flagella are correlated with cell responses and sensitivity to the surrounding environment, are self-sustained, and yet, are stable in the presence of small to moderate biochemical and background fluctuations (Wan and Goldstein 2014). Synchronization mainly occurs according to two stereotypical ways: the first is present in biflagellated algae such as *Chlamydomonas*. This alga is a typical "puller", in which propulsion acts from the front of the swimmer by the breaststroke-like beating of its pair of anterior flagella. The two flagella beat synchronously with identical frequencies and phases (Kurtuldu et al. 2013). The second way is present in multiflagellated algae, such as *Volvox* colonies. The flagella exhibit metachronal waves in which flagellar phases vary monotonically with position (Brumley et al. 2015).

In this paper we will try to elucidate the swimming behavior of *Tetraflagellochloris mauritanica*, a unicellular-multicellular chlamydomonadales. This alga possesses peculiar characteristics i.e. the cell body is bilaterally symmetric, the eyespot is located on the antero-posterior axis (anterior end of the cell), opposite to the flagella insertion (rear end of the cell), and two of the four flagella are considerably longer than the other two, in contrast to chlamydomonadales, in which the position of the eyespot gives the algae distinctive left-right asymmetry and flagella have equal length. This quadriflagellate alga has a complex swimming motion consisting of alternating swimming phases connected by in-place random reorientations and resting phases. It is capable of backward (ciliary beating) and forward swimming (flagellar beating), both being normal modes of swimming. The complex swimming movement resembles the run-and-tumble motion of peritrichous bacteria, with in-place reorientation taking the place of tumbles. During forward swimming *T. mauritanica* moves like a pusher, i.e. the cell has a positive force dipole and induces a flow field directed away from the cell along the swimming direction. The flagellar beat is very efficient, with undulatory retrograde waves originating at the

proximal-base portion of the flagella and running along them to the tip. During backward swimming the cell moves like a puller, i.e. it has a negative force dipole and induces an attractive flow field along the swimming direction. A non-stereotypical synchronization mode between flagella is present, with a pattern that does not fit any of the modes present in other Chlamydomonadales.

Material and Methods

Algal Cultures

Tetraflagellochloris mauritanica L.Barsanti & A.Barsanti cells were isolated by one of us (L.B.) from an environmental sample collected from a saline dry basin in the iron mining area of F'derick, in Mauritania, on November 2002. Cells were grown as non-clonal cultures in modified Johnson's medium at 24°C (dynamic viscosity 0.96 x 10^{-3} $m^{-2} \cdot s^{-1}$), and a 12:12 h L:D cycle (150 µmol photons $\cdot m^{-2} \cdot s^{-1}$; Barsanti et al. 2013).

Imaging Tetraflagellochloris Swimming

Cells were harvested from 4-d-old cultures at a density of about 10^5 cells $\cdot mL^{-1}$. For recording, 20 µL culture were placed between an acid washed slide and a 20 x 20 mm coverslip separated by a 100 µm shim spacer for optimal motility and to avoid hydrodynamic surface constraints, and viewed under a Zeiss Axioplan microscope (Carl Zeiss AG, Germany) equipped with bright-field 20x, 40x and 100x Plan-Apo objectives (Carl Zeiss AG, Germany).

Video microscopy was performed at 500 fps and at 512x512 pixels resolution (Fastcam SA3, Photron, U.S.). These time and spatial resolutions are well suited to observe the travelling wave propagating tip ward along the flagella (Wan and Goldstein, 2014). The projected position and orientation of the cell body as well as the shape and position of the flagella were obtained from high-speed recordings. The phototactic behavior of *T. mauritanica* has not been investigated; hence its spectral sensitivity distribution and threshold are unknown. Therefore, the uniformly distributed microscope light (100 W tungsten lamp) was filtered through neutral density filters (Knight Optical, UK) to obtain a light intensity higher than the camera sensitivity (10^4 ISO Ssat 12232 standard) and to avoid any kind of taxis during the experiments. Data from thousands of cells were analyzed.

Swimming efficiency computation

Swimming efficiency is computed for the forward motion of both single cells and colonies. As already stated, swimming efficiency can be defined as the power consumption to swim a certain distance compared with the power one would need to drag the body through the fluid. To calculate it, we adapted the model swimmer and

computation mode used by Tam and Hosoi (2011) for uniflagellated spermatozoa. For these authors, the swimming efficiency associated with a given stroke can be defined as the ratio between the power required to translate the payload (the head of the spermatozoon) at a certain average speed with a certain drag coefficient, and the average mechanical power dissipated through viscosity during one stroke. The swimming efficiency depends on the stroke kinematics and two dimensionless parameters characterizing the geometry of the swimmer, i.e. the flagellum-to-body lengths ratio $\boldsymbol{\eta = L_f / d}$, and the inverse slenderness of the flagellum $\boldsymbol{k = L_f / r}$, where $\boldsymbol{L_f}$ is the flagellum length, $\boldsymbol{d}$ is the cell diameter of the cell body approximated by a rigid sphere as in Tam and Hosoi (2011), and $\boldsymbol{r}$ is the flagellum radius. In our model, the diameter $\boldsymbol{d}$ of the sphere ranges from 3 µm (single cell) to 15 µm (16 cells). The 4 flagella of *Tetraflagellochloris* are modelled as a single whip, because during forward swimming they are perfectly phase-locked. The whip moves in a planar wave in the case of single cells and in a three-dimensional wave in the case of colonies; it is described by a set of 9 arcs (with 8 nodes) of equal length and amplitude of the wave decreasing from the insertion to the tip of the flagella (Lauga and Eloy 2013). The whip has a length $\boldsymbol{L_f}$ of 36 µm, corresponding to the length of the longer flagella; in the case of single cells, the whip radius $\boldsymbol{r}$ is 0.4 µm for the first 3 arcs (i.e., when the flagella are 4), and 0.2 µm for the other 6 arcs (i.e. when the flagella are 2, only the longer pair). In the case of colonies, the radius increases by 0.4 µm for the first 3 arcs, and 0.2 µm for the other 6 arcs every cell added. To perform swimming efficiency computations, three features for each segmented cells were extracted from time-lapse sequences, i.e., position in the plane (x, y coordinates of the barycenter), orientation angle $\boldsymbol{\alpha}$ of the cell body axis respect to the swimming direction in case of single cells, or rotation angle β around the swimming direction for the colonies. These two rotations are mutually exclusive. Features extraction was performed by means of MATLAB software routines, using algorithms previously described (Coltelli et al. 2013, Coltelli et al. 2014).

Flagellar synchronization computation

To perform this computation, five parameters for each segmented cell were extracted from the time-lapse sequence: position in the plane (x, y coordinates of the barycenter), orientation angle α of the cell body axis respect to the swimming direction, and two flagellar angle γ_l and γ_r for the left and right flagella couples, respectively. Features extraction was performed by means of MATLAB software routines, using algorithms previously described (Coltelli et al. 2013; Coltelli et al. 2014).

To monitor biflagellar synchrony dynamic, the phase difference variation, i.e., $\Delta\varphi_{l\text{-}r} = \varphi_l(t) - \varphi_r(t)$, is of particular interest (Wan and Goldstein, 2014), since the beating of each flagellar couple is characterized by a single periodic phase variable $\varphi_l(t)$ and $\varphi_r(t)$. The synchrony dynamic is said to be phase-locked if the phase

difference variation between the flagellar couples is constant. The synchrony dynamic is not phase-locked if the phase difference varies periodically or aperiodically. Flagellar synchronization is analyzed only in the backward swimming of single cells, because during forward swimming the four flagella of *T. mauritanica* are perfectly phase-locked behind the cell and beat synchronously, and uni-directionally.

The bilateral geometric disposition of the two flagella couples of *T. mauritanica* facilitates the extraction of phases for a couple's oscillations during backward swimming, and in turn, the derivation of phase synchrony dynamic between the coupled pairs. To calculate the synchrony variation ($\Delta\varphi_{l\text{-}r}$), we measured the γ angles between the tangent at the flagella in the resting position and the tangent at the flagella during beating. The γ value determines the correspondent phase value. The difference between phase values of the left and right flagella couples ($\varphi_l(t) - \varphi_r(t)$) is the synchrony variation.

Results

Anatomy

Tetraflagellochloris mauritanica (Fig. 1a) has a symmetrical ellipsoidal cell body, marked by the eyespot position in the anterior portion of the cell. It can be found as single tetraflagellated cell, but can also form non-coenobial colonies, which can consist of up to 16 (2^4) cells, with a number of flagella that is multiple of 4 (Fig 1, b and c). Sporadically, also colonies with an odd numbers of cells can be observed. Cells are kept together by some sort of diaphragm, and by interconnecting bridges similar to the cytoplasmatic bridges of *Volvox* (Hoops et al. 2005).

Cells are 3–5 μm long and about 2–2.5 μm wide. They possess four acronematic flagella: the two shorter and equal flagella are 11–12 μm long and the two longer and equal flagella are about 33–36 μm long. In the resting position the flagella are typically geniculate, with a sharp bend about 1 μm from the point of emergence; the angle is about 45° from the longitudinal cell axis (Fig. 1d). In this position, due to the length of their flagella and the surface tension, either single cells or colonies can float, without sinking, up to minutes.

Overall swimming

The overall motion is quite complex, since this quadriflagellate is capable of both swimming and floating in resting position. The complex swimming motion of a single cell consists of rapid phases of forward swimming of variable length along a straight path, which is followed by in-place random reorientations, and/or by phases of slow backward swimming, which can reverse the swimming direction. An example of this motion is shown in Figure 2a. In Figure 2b the motion phases are shown in a space versus time plot. Backward and forward

swimming are both normal mode of swimming, and backward swimming is not a reaction to a light level change, as in other biflagellated algae as *Chlamydomonas*. The transition between the phases as well as the beginning and end of the motion are marked by the flagella in the bent configuration of the cell resting position (Figure 1d).

During forward swimming, cells move like "pushers"; the four rear-mounted flagella beat synchronously with a frequency of about 10±1 Hz, uni-directionally, and perfectly phase-locked behind the cell; undulatory retrograde waves are produced at the base-proximal part of the flagella and run along them to the tip.

During backward swimming, cells move like "pullers"; the right and left flagella couples beat asynchronously alternatively and sequentially every 400 ms, that is, one couple of flagella has finished one effective beat before the other couple enters the effective beat of its next cycle. Each single beat of a flagella couple contains two components: the power stroke and the recovery stroke, the former faster (about 80 ms) than the latter (about 120 ms). Moreover, during the power stroke, the initiation of the wave is faster than its propagation to the tip, where the velocity slows down. The flagella then extend completely before curving closer to the cell body in the recovery movement. In the backward swimming the cells follow an almost straight path with a velocity that is positive during both the power stroke and the recovery stroke.

The forward swimming followed by resting phases is the only mode of swimming of the colonies. All the sequences begin with the cells in their resting configuration, with the flagella extended laterally in the typical geniculate position.

The speed of single cells ranges from 260 to 350 $\mu m \cdot s^{-1}$ (mean 300 SD.±35 $\mu m \cdot s^{-1}$) during the forward motion, and slows down to a range from 85 to 120 $\mu m \cdot s^{-1}$ (mean 102 SD.±13 $\mu m \cdot s^{-1}$) when the cells move backward. The speed of forward swimming colonies ranges from 83 to 115 $\mu m \cdot s^{-1}$ (mean 98 SD.±11 $\mu m \cdot s^{-1}$).

Figure 3 shows the distribution of time of forward swimming events (red dots), backward swimming events (blue triangles), in-place reorientations (empty red dots). All the distributions are well described by an exponential decay with time constants of 2.3 s, 1.4 s and 0.4 s respectively, suggesting an underlying Poisson process (Polin et al. 2009).

The time spent in the three phases of the swimming, excluding the time spent in the resting phase, is partitioned as in the following: ≈ 70% forward swimming; ≈ 20% backward swimming; ≈ 10% in-place-reorientation.

Figure 4 shows a sampling of shapes from the continuous motion of *T. mauritanica*: forward and backward movement of a single cell (sequences **a, b**), forward movement of a couple (sequence **c**) and forward movement of a colony (sequence **d**). Only the most representative shapes are shown. In this figure, flagella appear shorter than their real length because their beating causes the tips to leave the focal plane during the recording.

To swim forward (sequence **a**), the cell initiates the motion by pushing the body forward and simultaneously throwing the flagella behind in an arched concave position. As the cell proceeds, the four flagella gain an extended shape with a marked reduction of the curvature, which becomes almost convex, and join to get ready for the flagellar beating. Undulatory waves then initiate at the base-proximal part of the flagella and run along them to the tip to propel the cell forward. During forward swimming, the cell body rocks around the swimming direction axis. At the end of the swimming phase, the cell slows down and undulatory waves stop because the flagella begin to separate in the proximal portion. The separation proceeds and involves also the distal portion of the flagella, which gain again the arched concave position, with the flagellar tips facing toward the axis of symmetry of the cell. The definite stop occurs when the cell body hinges onto the flagellar insertion to rotate backward; the result of this flipping is a resting configuration that mirrors that of the first frame.

Backward swimming (sequence **b**) can initiate either from the resting position or from forward swimming without flipping. During this motion, the right and left flagella couples alternate their beating in sequence: the movement of a couple begins when the movement of the other couple ceases. Also in this case, the resting configuration marks the end of the motion.

In the case of a cell couple (sequence **c**) forward swimming retraces the same steps of the single cell motion, from a resting position to a mirror resting position, gained by flipping of the cell bodies onto the flagellar insertion. However, the couple, while swimming, rotates around its axis of symmetry instead of rocking. The colonies move in a similar way, beginning from the resting position, and proceeding rotating around their axis of symmetry, with a frequency of about 2 Hz. In this case, the resting position at the end of the motion is the same of the beginning, i.e. the colonies cannot flip.

Swimming efficiency computation

Figure 5 shows the result of the computation procedure similar to that used by Tam and Hosoi (2011) to calculate the parameters of an optimal stroke and its efficiency. Among the different configurations of cell geometries obtained keeping the flagella length $\boldsymbol{L_f}$ fixed and varying the body diameter $\boldsymbol{d}$, the flagellar bundle radius $\boldsymbol{r}$, and the orientation angle $\boldsymbol{\alpha}$ of the cell body, the representative optimized swimmer resulting from the

computation possesses a wavelength λ equal to 30 µm, a maximal wave amplitude A equal to 3 µm, an optimal flagellum-to-body lengths ratio η of about 12, and an inverse slenderness of the flagellum k of about 180 (Fig. 5a).

Figure 5b show an example of time sequence of optimal strokes for a single cell. In this case, the calculated rocking angle α of the cell body axis with respect to the swimming direction varies periodically from -20° to + 20°, and its variation is almost identical to that of the measured rocking angle, (Fig. 5c).

The computation of the efficiency ε of *T. mauritania* stroke confirmed the fact that its anatomical parameters are optimal for swimming. ε value is very high in the case of single cells, about 1.4%, and lowers to 0.8% in the case of colonies because of the decrease of both η and k, due to higher d and r values.

Flagellar synchronization computation

Figure 6a shows the time history of the flagellar beating during the asynchronous beat cycle of the backward swimming. Only the beating of the left flagella couple is shown, since the two couple beats alternatively and sequentially mirroring each other motion. Either flagella couple can start the swimming. The angular values, indicated in the figure, represents the γ angles measured between the tangent at the flagella in the resting position and the tangent at the flagella during beating. The beating of each flagella couple is characterized by a single periodic phase φ (t), whose value is indicated in correspondence of the 9 flagellar shapes, (1 resting position + 8 beating positions). While one flagella couple completes its beat cycle, the other couple (the right one in Fig. 6a) is temporarily motionless. Figure 6b shows the saw tooth plot of γ_l angle and the stationary plot of γ_r angle during the beat cycle. We recorded an alternative and sequential flagellar beating with a flagellar phase difference $\Delta\varphi_{l\text{-}r}$ ranging from 0 to 2π. The two flagella couples maintains this behavior during free swimming and phase-slip asynchronies, associated with rapid changes in inter-flagellar phase difference, were never observed.

Figure 7a shows an asynchronous beat cycle of the left flagella couple; for the sake of clarity only 7 of the 9 flagella positions are shown. The eyespot end of the cell body rocks toward the side opposite to the side of the moving flagella couple, i.e., counterclockwise. The cell swims 20 µm backward during each beat cycle of 200 ms, and rocks with an angle α that varies from 0° to 20° (Fig. 7b).

Discussion

Anatomy

Tetraflagellochloris mauritanica separates at a basal ancestral position with respect to the entire Chlamydomonadales lineage; the most outstanding features that differentiate it from the other Chlamydomonadales are the following: this alga can grow as single cells or as non-coenobial colonies with up to 16 individuals, without germ-soma division of labor; it possesses four independent flagella of different lengths, two longer and two shorter, and four independent basal bodies arranged in a diamond configuration; two basal bodies are arranged in a V-shaped configuration, while the other two are arranged in a parallel configuration; grouped cells show an unusual but well-ordered arrangement of flagella (Fig. 1) (Barsanti et al. 2013).

Though Chlamydomonadales are considered good swimmers (Goldstein 2014), we can say that the *T. mauritanica* single cell is an exceptional swimmer having an extraordinary effective flagella apparatus as it has a double set of motors and an increased length relative to slower competitors. In this alga, the flagellum-to-body lengths ratio $\boldsymbol{\eta}$ varies from about 12 for 1 cell to about 2.4 for 16 cells and the inverse slenderness of the flagellum $\boldsymbol{k}$ varies from about 90 for 1 cell to about 5.6 for 16 cells. On average, Chlamydomonadales have $\boldsymbol{\eta}$ and $\boldsymbol{k}$ lower than a 16 cells *T. mauritanica* colony, suggesting that the flagellar apparatus of this alga is well suited not just for a single cell, but also for a multiple cell organization.

Overall swimming

We have shown that the non-stimulated movement of *T. mauritanica* cells is the result of stochastically switching over the course of time between three swimming modes (Figs. 2 and 3): forward synchronous mode, backward asynchronous mode and in-place reorientation mode. Forward swimming and in-place reorientations are common to single cells and colonies, whereas backward swimming is a characteristic of single cells. As far as we know *T. mauritanica* is the only Chlamydomonadales able to switch between forward swimming, performed as a pusher, and backward swimming, performed as a puller, without any external stimuli. Usually the change of the swimming direction is an avoiding reaction to external stimuli (Inouye and Hori 1991).

The forward swimming mode of *T. mauritanica* cells consists of planar and undulatory waves that originate from the base-proximal part of the flagella and travel along their length to the tip (Cohen and Boyle 2009).

According to Inouye and Hori (1991) this swimming behavior can be considered quite primitive since it resembles the movement of a bacterium, a typical symmetric pusher, though bacteria have a completely different motility system.

The beating frequency of *T. mauritanica* (10 Hz) swimming as a pusher is equal/lower than other Chloropyta such as *Pterosperma cristatum* (10 Hz) and *Cymbomonas tetramitiformis* (40 Hz), which swim in a similar manner (Inouye and Hori 1991); this can be due to the length of the flagella and the wavelength of the traveling wave (Fig. 5). Single cell velocity is very high, about 300 $\mu m \cdot s^{-1}$, and therefore we can rank *T. mauritanica* among the fastest algae (Barsanti and Gualtieri 2014).

When *T. mauritanica* swims as a puller (Figs. 6 and 7), beating frequency is much lower (2.5 Hz) than that of *Chlamydomonas*, (50-60 Hz; Leptos et al. 2013) and single cell velocity is always positive, during both the recovery stroke (from 0 to π in Fig. 6a) and the power stroke (from π to 2 π in Fig. 6a). A possible explanation of these positive velocities could be the residual forward thrust left by the body rotation. However, due to the different time scale, the distances traveled are different (Fig. 7a).

Tetraflagellochloris mauritanica swimming mode differentiates from that of other algae due to:

1) the stochastic pattern of its overall swimming that resembles the run-and-tumble motion of peritrichous bacteria, with in-place reorientation taking the place of tumbles, in a way similar to picoplankton;
2) the capacity to floating thanks to surface tension because of the length and geniculate position of its flagella couples, without any aid from buoyancy devices such as gas filled vacuoles or oil droplets.

Polin et al. (2009) already suggested a similar stochastic swimming strategy for *Chlamydomonas,* termed "run and turn" locomotion. The difference is that the changing of direction in *Chlamydomonas* is obtained only by sharp turns quite different from tumbling, which require a sophisticated "two gear" steering mechanism; in *T. mauritanica*, in-place reorientation really resembles bacterial tumbling and picoplankton behavior.

Swimming efficiency

The velocity of forward movement is always a small fraction of the velocity of the wave running along the flagellum, and its propulsive efficiency $\boldsymbol{\varepsilon}$ mainly depends on wavelength $\boldsymbol{\lambda}$, amplitude $\boldsymbol{A}$, $\boldsymbol{\eta}$ and $\boldsymbol{k}$, which in turn depend on flagellum length $\boldsymbol{L_f}$ and cell body diameter $\boldsymbol{d}$. As the length of the flagella approaches zero, the swimmer can be considered a single rigid sphere that cannot longer swim; hence its swimming efficiency is zero. Similarly, as the length of the flagella approaches to infinity, most of the mechanical power is used to

overcome the drag on the flagella, and again the efficiency associated with transport of the cell body decays to zero. Between these two extremes, strokes with efficiencies greater than zero must exist.

To find the optimal parameters we computed the optimal stroke kinematics for different configuration of cell geometries. We selected head diameters $\boldsymbol{d}$ ranging from 3 to 15 μm and fixed flagella length and radius ($\boldsymbol{L}_f$ =36 μm, $\boldsymbol{r}$ =0.4 μm). We found that, for a given head size, optimal values of $\boldsymbol{\eta}$ exist, consistent with previous work, which investigated swimmers using prescribed suboptimal sinusoidal strokes (Higdon 1979, Tam and Hosoi 2011).

To explore the space of all acceptable kinematics, the optimization procedure started from a wide range of initial strokes, which varied in structure, symmetry, and amplitude. The solution the optimization procedure converged to, consists of symmetrical undulatory bending waves with localized regions of high curvature, which form at the base-proximal part of the flagella and propagate toward their end. A representative computed optimized swimmer is shown in Figure 5a, with $\boldsymbol{d}$ = 3 μm and $\boldsymbol{L}_f$ = 36 μm. Figure 5b represents superposed snapshots of the optimal stroke, which shows rocking angle variation almost identical to that recorded (Fig. 5c). Hence, the undulatory nature exhibited by this optimal stroke is in qualitative and quantitative agreement with strokes observed in single cells of *T. mauritanica*, which possesses the perfect anatomy to swim as a pusher.

The efficiency $\boldsymbol{\varepsilon}$ of this optimal stroke is about 1.4%, which corresponds to an increase of about 70% over the maximum efficiency of 0.8% attained by the breaststroke of biflagellated algae, such as *Chlamydomonas* (Guasto et al. 2012). For this optimal efficiency, the value of $\boldsymbol{\eta}$ was calculated to be about 12, the same value of *T. mauritanica*; since this value relies only on purely hydrodynamic considerations, it should be a common feature of optimal microswimmers. Colonies reach 0.8% efficiency confirming that the flagellar apparatus is well suited not just for a single cell, but also for a multiple cell organization.

Flagellar synchronization

The coordination of eukaryotic flagella is essential for many of the most basic processes of life (motility, sensing, development, and evolution of multicellularity; Solari et al. 2006), hence, its regulation and connection to locomotion are more and more investigated, and the more sustained hypothesis implicates hydrodynamic interactions between flagella (Golestanian et al. 2011, Uchida and Golestanian 2011, Geyer et al. 2013, Brumley et al. 2014).

The mechanism of synchronization necessitates of interflagellar coupling, which can be obtained by the motion of the fluid medium surrounding the cell, by rocking of the cell body, or by internal modulation of the elastic components connecting the basal bodies (Lechterck and Melkonian 1991). A possible explanation of the mechanisms of synchronization based on hydrodynamic interactions is the following (Wan et al. 2014). For in-phase synchronization, the flagellar beat is mirror-symmetric and the cell swims along a straight path. If, however, the left flagellum has a small head start during the effective stroke, this causes a counter-clockwise rotation of the cell. This cell-body rocking increases the hydrodynamic friction encountered by the left flagellum, causing the left flagellum to beat slower and the right one to beat faster, and flagellar synchrony is restored. Cell body rocking can occur also on the right side.

During forward swimming, the four flagella of *T. mauritanica* beat synchronously, uni-directionally, and perfectly phase-locked behind the cell; undulatory retrograde waves are produce at the base-proximal part of the flagella and run along them to the tip, in a flagella-like beating, without any asynchronic episode, (figure 5).

When *T. mauritanica* swims backward the flagella beat with time-shifted mirror symmetry and a phase variation from 0 to 2π, (Fig. 6, a and b). This swimming mode is quite uncommon among unicellular algae and it is not a transitory effect as a slip, but it is a true gait. In our case, the observed cell body rocking during backward swimming, (Fig. 7, a and b), and the consequent differential change in hydrodynamic friction are not enough to restore the synchronicity between the two flagella couples, due to their length and mass and the small size of the cell body.

The mode of coordination of *T. mauritanica* can be described qualitatively as “serial coordination”, where flagella couple beat alternatively and sequentially, different from the mode of synchronization of ptx1 *Chlamydomonas* mutant, described as ‘‘parallel coordination’’ (Ruffer and Nultsch 1998) where flagella lock in antiphase synchronization, and from the mode of synchronization of wild type *Chlamydomonas* described as ‘‘bilateral coordination’’, where flagella are synchronized (Leptos et al. 2013).

Figure Captions

Figure 1

SEM micrograph of a single *Tetraflagellochloris mauritanica* cell showing the four flagella, scale bar: 3 µm; b) SEM micrograph of a couple showing the arrangement of the 8 flagella, scale bar: 1 µm; c) SEM micrograph of a colony, scale bar: 5 µm; d) light microscope image of a single cell in the resting position with the flagella in the bent configuration, scale bar: 4 µm.

Figure 2

a) Tracked swimming motion of a single cell of *Tetraflagellochloris mauritanica* showing the three different phases: forward swimming (1-2, 3b-4a, 4b-5a, 5b-6a, 6b-7); backward swimming (2-3a); in-place reorientation (3a-3b, 4a-4b, 5a-5b, 6a-6b). b) The phases are shown in a space versus time plot.

Figure 3

Distribution of times between the three phases of swimming: forward swimming (red dots); backward swimming (blue triangles); in place reorientation (empty red dots).

Figure 4

All the possible shapes of the different swimming movements of *T. mauritanica*: forward and backward movement of a single cell (sequences a, b), forward movement of a couple (sequence c) and forward movement of a colony (sequence d). Only the most representative shapes are shown. The green traffic light indicates the start of the swimming sequence, while the chequered flag indicates the end of the sequence.

Figure 5

Result of the swimming efficiency computation. a) Model swimmer: all the parameters and the 9 arcs are shown; b) time sequence of a single cell optimal strokes; c) rocking angle variation for the optimized swimmer (red line) and for *T. mauritanica* (blue circles, blue line).

Figure 6

Asynchronous beat cycle of a single cell during backward swimming. a) Time history of the beating of the left flagella couple: the angular values represent the γ_l angles between the tangent at the flagella in the resting position and the tangent at the flagella in the beating position; the red positions belong to the recovery stroke, while the green positions belong to the power stroke; b) the saw tooth plot of the γ_l angle and the stationary plot of the γ_r angle versus time.

Figure 7

a) An asynchronous beat cycle of the left flagella couples: for the sake of clarity only 7 of the 9 flagella positions are shown; the cell swims 20 μm backward during a cycle of 200 ms; b) the cell rocks with an angle α that varies from 0° to 20°; all the 9 rocking angles are shown.

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