Fidelity of adaptive phototaxis

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Along the evolutionary path from single cells to multicellular organisms with a central nervous system are species of intermediate complexity that move in ways suggesting high-level coordination, yet have none. Instead, organisms of this type possess many autonomous cells endowed with programs that have evolved to achieve concerted responses to environmental stimuli. Here experiment and theory are used to develop a quantitative understanding of how cells of such organisms coordinate to achieve phototaxis, by using the colonial alga Volvox carteri as a model. It is shown that the surface somatic cells act as individuals but are orchestrated by their relative position in the spherical extracellular matrix and their common photosresponse function to achieve colony-level coordination. Analysis of models that range from the minimal to the biologically faithful shows that, because the flagellar beating displays an adaptive down-regulation in response to light, the colony needs to spin around its swimming direction and that the response kinetics and natural spinning frequency of the colony appear to be mutually tuned to give the maximum photosresponse. These models further predict that the phototactic ability decreases dramatically when the colony does not spin at its natural frequency, a result confirmed by phototaxis assays in which colony rotation was slowed by increasing the fluid viscosity.

adaptation | evolution | flagella | fluid dynamics | multicellularity

The most primitive “eyes” evolved long before brains and even before the simplest forms of nervous system organization appeared on Earth (1, 2). Many organisms are able to sense and respond to light stimuli, an ability essential to the optimization of photosynthesis, the avoidance of photodamage, and the use of light as a regulatory signal. One of the more striking responses is phototaxis, in which motile photosynthetic microorganisms adjust their swimming path with respect to incident light in a finely tuned manner (3, 4). This steering relies on sensory inputs from one or more eyespots (2), primitive photosensors that are among the simplest and most common “eyes” in nature. They consist of photoreceptor proteins and an optical system of varying complexity, which provide information about the intensity and directionality of the incident light (2, 3, 5). This information is then translated into an organism-specific swimming control mechanism that allows orientation to the light with high fidelity.

In most unicellular phototactic organisms, such as the archetypal green alga Chlamydomonas, the presence of a single eyespot implies both a limited vision of the three-dimensional world in which the cell navigates and the impossibility of detecting light directions by measuring light intensity at two different positions in the cell body. To overcome these restrictions, such organisms must compare light intensity measurements from their single eyespot at different moments in time (6). Many species do this by swimming on helical paths along which their eyespot acts as a light antenna continuously searching space for bright spots (3). Higher eukaryotes have a nervous system to integrate visual information from different sources and orchestrate coordinated responses (7, 8).

Multicellular organisms of intermediate complexity, such as the colonial alga Volvox and its relatives (9), have evolved a means of high-fidelity phototaxis without a central nervous system and, in many cases, even in the absence of intercellular communication through cytoplasmic connections (10). Volvox carteri consists of thousands of biflagellated Chlamydomonas-like somatic cells sparsely distributed at the surface of a passive spherical extracellular matrix, and a small number of germ cells inside the sphere (Fig. 1A). During development the flagella orient such that Volvox rotates about its swimming direction, the trait that gave Volvox its name (11). Coordination of the somatic cells resembles orchestrating a rowboat with thousands of independent rowers but without a coxswain (9). Nature’s solution is a response program at the single-cell level that produces an accurate steering mechanism, an emergent property at the colonial level. Yet it remains to be understood what form the response program must take to coordinate the cells and to yield high-fidelity phototaxis in the presence of the steering constraints of a viscous environment.

More than a century ago, Holmes (12) proposed that the somatic cells facing a source of light down-regulate their flagellar activity, a hypothesis later confirmed by several investigators (13–16). Although this control principle will initially turn the colony towards the light, the colony might adapt (14, 15) to the light before good alignment with the light direction has been reached. Surprisingly, this observation has not been synthesized into a predictive, quantitative model consistent with the principles of fluid dynamics, nor are there data on Volvox phototaxis that can be compared with such a theory. Here we use a combination of experiment and theory to show that adaptation and colony rotation play key roles in the phototaxis mechanism of V. carteri. By quantifying the flagellar photosresponse of V. carteri in detail, we show that it acts as a band pass filter that allows adaptation to different light environments, minimizes the influence of fast light fluctuations, and maximizes the response to stimuli at frequencies that correspond to the rotation rate of the organism. These measurements suggest that the response kinetics and colony rotation have evolved to be mutually tuned and optimized for phototaxis. Furthermore, we develop a mathematical theory that predicts the phototactic fidelity of Volvox as the rotation rate and other parameters change and confirm experimentally that colony rotation is essential for accurate phototaxis.

Results and Discussion

Temporal Dynamics of the Adaptive Response. The most elementary photosresponse is the change in flagellar beating accompanying a step up or down in illumination intensity. This response is probed with the experimental setup shown in Fig. 1B. Cyan light from an LED that is coupled to a fiber-optic light guide held in a micropipette is directed toward the anterior of a V. carteri colony held by a second micropipette. Details are given in Materials and Methods and SI Text. High-speed imaging of flagella revealed, in accord with proposals by several investigators (13–15), that the somatic cells change their beating frequency rather than their flagellar activity, 17]. Instead of quantifying the average photosresponse by recording the beating frequency of each flagellum of

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every somatic cell, we measured the fluid motion produced by the flagellar beating by using particle image velocimetry (PIV). This approach implicitly averages over several neighboring flagella, and, by measuring the fluid velocity just above the flagellar tips, we obtain a natural input for the hydrodynamic models of photosynthetic turning described further below. Because of the low Reynolds number associated with flows generated by \( V.\ carteri \) (18–20), fluid inertia is negligible and the flagella-induced flow is a direct measure of the flagellar activity. Fig. 2A shows a typical time trace of the flagellar photoresponse, in terms of the flagella-generated fluid speed \( u(t) \), normalized by the flow speed under time-independent illumination \( u_\text{on} \), and averaged over \( \pm 30^\circ \) from the anterior pole. We found that a step up in light intensity elicits a decrease in flagellar activity on a response time scale \( \tau_r \), followed by a recovery to baseline activity on a time scale \( \tau_a \) associated with adaptation; there was no change in flagellar activity upon a step down in light intensity. This response underlies the ability of \( V.\ carteri \) to turn toward the light, as explained further below. At very high light intensities and long stimulation, the responses to step up and step down stimuli are reversed (see SI Text), allowing \( V.\ carteri \) to avoid photodamage by swimming away from the light. Irrespective of the stimulus light intensity, \( \tau_r \) is always a fraction of a second, whereas \( \tau_a \) is several seconds (Fig. 2B), consistent with early observations (14, 15).

Although the kinetics and biochemistry of photoreceptor currents have been studied in \( Chlamydomonas \) (21, 22) and \( V.\ carteri \), their connection to the flagellar photoresponse is unclear. In \( Chlamydomonas \), a step stimulus elicits a \( \text{Ca}^{2+} \) current whose time scale of 1 ms (22) is too short to account for the measured \( \tau_r \). But the time for \( \text{Ca}^{2+} \) to diffuse the length of the flagellum \( L = L^2/D = 0.2 \) s (for \( L \sim 15 \mu\text{m}, D \sim 10^{-5} \text{cm}^2/\text{s} \)), which is similar to \( \tau_r \), suggesting that the photocurrent triggers an influx of \( \text{Ca}^{2+} \) at the base of the flagella, consistent with previous hypotheses (22, 23). Although the dependence of \( \tau_r \) on light intensity is like that of the \( \text{H}^+ \) current in \( V.\ carteri \), the decay constant of the latter is only \( \sim 75 \) ms (22); the biochemical origin of \( \tau_r \) remains unknown.

The measured adaptive response of the flagella-generated fluid speed just above the colony surface (Fig. 2A) can be described by \( u(t)/u_\text{on} = 1 - \beta \hat{p}(t), \) where \( \hat{p}(t) \) is a dimensionless photoreceptor variable that is large when there is a large light-induced decrease in flagellar activity and vanishes when there is no such change in flagellar activity. The empirically determined constant \( \beta > 0 \) quantifies the amplitude of the decrease in \( u(t)/u_\text{on} \). For a model of \( \hat{p}(t) \) that captures the two time scales \( \tau_a \) and \( \tau_r \), we require a second variable \( h(t) \), which we define as a dimensionless representation of the hidden internal biochemistry responsible for adaptation (24, 25). A system of coupled equations that is consistent with the measured \( u(t)/u_\text{on} \) is

\[
\begin{align*}
\tau_r \dot{h} &= (s - h)H(s - h) - p, \\
\tau_a \dot{p} &= s - h,
\end{align*}
\]

where the light stimulus \( s(t) \) is a dimensionless measure of the photoreceptor input that incorporates the eyespot directionality. The Heaviside step function \( H(s - h) \) is used to ensure that a step down in light stimulus cannot increase \( u \) above \( u_\text{on} \), because it keeps \( p > 0 \). In these equations, the values \( p^* = 0 \) and \( h^* = s^* \) are stable and global attractors in the sense that, after a sufficiently long time under constant light stimulus \( s(t) \), the pair \( (p, h) \) relaxes to \( (p^*, h^*) \). However, if \( s \) increases from \( s_1 \) for \( t < 0 \) to \( s_2 \) for \( t \geq 0 \), then for \( t > 0 \) the solution is

\[
\begin{align*}
\dot{h}(t) &= s_1 e^{-t/\tau_a} + s_2(1 - e^{-t/\tau_a}), \\
\dot{p}(t) &= \left(\frac{s_1 - s_2}{1 - \tau_a/\tau_r}\right)(e^{-t/\tau_a} - e^{-t/\tau_r}).
\end{align*}
\]

When \( \tau_r \ll \tau_a \), as for \( V.\ carteri \), there is a sharp transient increase in \( p(t) \) [and decrease in \( u(t) \)], peaking at a time \( t = \tau_a \ln(\tau_r/\tau_a) \), followed by a slow relaxation back to zero, as in the measured flagellar photoresponse shown in Fig. 2A.

The rotation of \( V.\ carteri \) about its axis and the resulting periodic illumination of the photoreceptors suggest an investigation of the dependence of the photoresponse on the frequency of sinusoidal stimulation. For the above model this frequency dependence of the photoresponse is \( \mathcal{R} = \tilde{h}/\tilde{s} \), where \( \tilde{p} \) and \( \tilde{s} \) are the Fourier transforms of \( p \) and \( s \), respectively. \( \mathcal{R} \) is well-approximated by neglecting the Heaviside function in Eq. 1 (see SI Text) to give

\[
\mathcal{R}(\alpha_s) = \frac{\alpha_s^2 \tau_a}{\sqrt{1 + \alpha_s^2 \tau_a^2}(1 + \alpha_r^2 \tau_a^2)}.
\]
If the stimulus angular frequency $\omega_r$ is very low ($\omega_r \ll 2\pi/\tau_S$), the adaptive process has sufficient time to keep up with the changing light levels and the amplitude of the response vanishes. At very high frequencies, $\omega_r \gg 2\pi/\tau_S$, the response is limited to short-time behavior and also vanishes.

Using the setup in Fig. 1B, we measured the flagellar photoreponse to sinusoidal light stimuli of various temporal frequencies. In Fig. 3A, these measurements are compared with the theoretical $R(\omega_r)$, showing excellent agreement. The maximum response is observed at stimulus frequencies that correspond to the natural angular rotation frequencies of Volvox about its swimming direction. The frequency dependence of the photoreponse is like a band pass filter that removes high frequency noise, e.g., light fluctuations from ripples on the water surface (26), but retains the key feature of adaptation.

**Heuristic Mechanism of Phototaxis.** We proceed to a qualitative discussion of how an adaptive response translates into phototactic turning and the ingredients required for a simple yet realistic mathematical model with predictive power.

In general, phototactic orientation is due to an asymmetry of the flagellar behavior between the illuminated and shaded sides of the organism. The mechanism that achieves this asymmetry is species-dependent, but it is instructive to consider a hierarchy of ingredients. First, consider a nonspinning spherical organism that can display a nonadaptive photoreponse on its entire surface but will do so only on the illuminated side, as in Fig. 4A. This organism will achieve perfect antialignment of its posterior-anterior axis $k$ with the light direction unit vector $I$ (i.e., face the light) on a turning time scale $\tau_k$ which is determined by the balance between the torques due to asymmetric flagellar activity and rotational viscous drag. Adaptation to light is a desirable property for such an organism, because it allows a response to light intensities over several orders of magnitude and because it allows the organism to swim at full speed once a good orientation has been reached. If the photoreponse of the above model organism now has the desirable property of being adaptive, it will initially turn towards the light as in Fig. 4A, but adaptation may cause the response to decay before $k$ reaches antialignment with $I$ (Fig. 4B), depending on the relative magnitude of $\tau_k$ and $\tau_I$. If, however, the adaptive organism would spin about $k$, new surface area would continuously be exposed, thus maintaining an asymmetric photoreponse until perfect antialignment of $k$ with $I$ has been reached. For Volvox, which generally have $\tau_k \sim \tau_I$, the spinning about the posterior-anterior axis is therefore not just optimized for the photoreponse kinetics, as shown in the previous section, but also essential for high-fidelity phototactic orientation in the presence of adaptation. Spinning may also mitigate the deleterious effects of unsymmetrical colony development and injury (27), and for organisms with a restricted field of view due to a small number of eyespots, such as Chlamydomonas and Platynereis, spinning is also required for detecting the light direction (3, 7).

In Volvox colonies, the flagellar photoreponse is localized near the anterior pole (Fig. 5), yet the importance of spinning outlined above remains. However, having only a small photoreponsive region complicates the heuristic picture: If the eyespots could only direct an all-or-nothing response as they move from the shaded to the illuminated side of the sphere, the best possible phototactic orientation is drawn in Fig. 4C. Such a mechanism...
of the thousands of individual flagella on the colony surface, we adopt a continuum approximation in which there is a temporally and spatially varying surface velocity. If $\theta$ and $\phi$ are the polar and azimuthal angles on a sphere, respectively, the surface velocity $\boldsymbol{u}$ may be decomposed into $\boldsymbol{u} = v\hat{\theta} + w\hat{\phi}$. We interpret $\boldsymbol{u}$ as the velocity at the edge of the flagellar layer (32); for practical reasons experimental measurements of $\boldsymbol{u}$ are made just above that layer. In the absence of a light stimulus, $\boldsymbol{u} = \boldsymbol{u}_0$ and we assume that the ratio $v_0(\theta)/w_0(\theta)$ is constant on the colony surface because of the precise orientational order of somatic cells (9).

Following step changes in light intensity, measurements of $v(\theta,\phi,t)$ at fixed $\phi$ show that in each region, the surface velocity displays a photosensitive form of the form shown in Fig. 2A but that the overall magnitude varies with $\theta$ (Fig. 5A).

We thus model $\boldsymbol{u}(\theta,\phi,t)$ by allowing the quantities $\beta$, $p$, and $h$ to depend on position:

$$\boldsymbol{u}(\theta,\phi,t) = \boldsymbol{u}_0(\theta)\{1 - \beta(\theta)p(\theta,\phi,t)\}.$$  

[6]

The measured $\beta(\theta)$ is shown in the inset in Fig. 5A.

To define the stimulus $s$ on the colony surface, we make use of the angle $\psi(\theta,\phi,\boldsymbol{I})$ defined through $\cos\psi = -\boldsymbol{n} \cdot \hat{\boldsymbol{I}}$, where $\hat{\boldsymbol{n}}$ is the unit normal to the surface. When $\psi = 0$ ($\pi$), the light is directly above (behind) a given surface patch. The light-shadow asymmetry in $s$ can therefore be modeled by a factor $H(\cos\psi)$. Superimposed on this factor may be another functional dependence on $\psi$ to account for the eyespot sensitivity in the forward direction, with experiments on *Chlamydomonas* (28) supporting a dependence $f(\psi) = \cos\psi$. The class of models we consider for the dimensionless $s$ is therefore

$$s(\theta,\phi,\boldsymbol{I}) = f(\psi)H(\cos\psi).$$  

[7]

With the above specification of the dynamics of the surface velocity, the angular velocity of the colony is (31)

$$\Omega(t) = \frac{1}{\tau_{\text{bh}}} \boldsymbol{g} \times \dot{\boldsymbol{k}} - \frac{3}{8\pi R^3} \int \hat{\boldsymbol{n}} \times \boldsymbol{u}(\theta,\phi,t)dS,$$  

[8]

where $\hat{\boldsymbol{g}}$ and $\hat{\boldsymbol{k}}$ are the directions of gravity and the posterior-anterior axis, respectively. The first term in Eq. 8 arises from bottom-heavyness and represents a balance between the torque that acts when the posterior-anterior axis is not parallel to gravity and the rotational drag of the sphere (20). The second term is responsible for phototactic steering, where the integral is taken over the surface of the sphere of radius $R$. In a reference frame where the Volvox is at the origin with a fixed orientation, the light direction evolves as $\delta \boldsymbol{I}/dt = -\Omega \times \boldsymbol{I}$.

The above coupled equations can be solved numerically (see SI Text), e.g., to determine the angle $\alpha(t)$ of the organism axis with the light direction. It is interesting to consider two special cases of the model class outlined above. In the biologically faithful “full model,” we use the measured $\beta(\theta)$ and the realistic eyespot directionality $f(\psi) = \cos\psi$. In the “reduced model,” we consider only a light-shadow response asymmetry—i.e., $f(\psi) = 1$—and use the mean of the measured $\beta(\theta)$—i.e., $\beta(\theta) = 0.3$. All other features are shared between the models.

A phototactic turn of a hypothetical non-bottom-heavy Volvox simulated by the reduced model is shown in Fig. 6, indicating an intricate link between organism rotation, adaptation, and steering. In reality, however, Volvox is bottom-heavy, which is particularly important when the light direction is horizontal. In this case, we previously observed (33) that the organisms reach a final angle $\alpha_f$ set by the balance of the bottom-heavyness torque and the phototactic torque. We therefore define the “phototactic ability” $\alpha_f = (\text{swimming speed toward the light})/(\text{swimming speed})$.

Both models predict that as the viscosity $\eta$ is increased, while keeping the internal parameters $\tau_1$ and $\tau_2$ fixed, the phototactic ability decreases dramatically (Fig. 7). Qualitatively, an increase in $\eta$ reduces $\alpha_f$, which leads to a reduced phototactic response (Fig. 3A).
and therefore a reduced phototactic torque. The sharp transition in Fig. 7 occurs when the phototactic torque becomes comparable to the other torques in the system. The simulations neglected torques due to ambient fluid motion and included only the bottom-heavyness torque.

We tested the above prediction by measuring the phototactic ability of *Volvox* at various viscosities in a population assay at low organism concentration and negligible ambient fluid motion. It is important to note that, in the experiment, the phototactic ability is a measure of a slightly different quantity than in the model. In the model, a bottom-heavy *Volvox* swims in an infinite fluid toward the light at an angle $\alpha_f$ with the horizontal. A colony swimming in the same direction in the experiment will collide with the top surface of the sample and change direction. The phototactic ability in the experiments is therefore a measure of the directionality of the population swimming behavior (see SI Text), whereas in the model it is solely a measure of $\alpha_f$. The data from several populations are shown in Fig. 7 and are found to be in quantitative agreement with the full model for realistic parameters (given in SI Text) and in qualitative agreement with the reduced model. The success of the reduced model highlights that spinning and adaptation are the key ingredients for a qualitative understanding of the fidelity of phototaxis in *Volvox* and that a quantitative understanding can be obtained if a realistic eyespot directionality and anterior-posterior response asymmetry are included. These models further illustrate that if all somatic cells were photoresponsive, the organism would have a higher phototactic ability (Fig. 7). Yet it may be beneficial to keep a high translation speed even during light stimulation, and there may be significant metabolic and developmental costs associated with endowing all cells with a photoresponse, which could make it advantageous to have the photoresponse localized in the anterior.

In additional experiments, we found that very large *Volvox* (Fig. 3B) have a much lower phototactic ability although they still display the flagellar photoresponse. For such colonies, the hydrodynamic model (Eq. 8) reveals that their lower phototactic ability arises from the increase in $R$ and concomitant decrease in $w_0$, and the reduction in phototactic $p$ due to the lower $\omega_0$ (Fig. 3A). The model thus yields insight into which parameters determine the phototactic torque and illustrates the intuitive result that this torque must be significantly larger than competing torques to achieve high-fidelity phototaxis.

**Conclusion**

We have shown how accurate phototaxis of the alga *V. carteri*, a colonial organism lacking a central nervous system, is achieved by autonomous cells on its anterior surface endowed with an adaptive flagellar photoresponse. The response and adaptation time scales of this phototaxis determine an optimal frequency for the characteristic spinning of *Volvox* about its swimming direction. Because the organisms naturally spin at this optimal frequency, the flagellar orientation and phototactic kinetics seem to have coevolved to maximize the phototactic ability. The mathematical model of phototaxis developed here shows that the phototactic fidelity decreases dramatically when the colony does not spin at its natural frequency; the results of a phototaxis assay in which spinning was slowed by increasing the fluid viscosity are in excellent agreement with the model predictions.

This work raises a number of issues for further investigation. Chief among them are the biochemical origin of the adaptive time scale and the reason for displaying a phototactic only in the anterior part of the organism. Because the rotational frequency of phototactically active *V. carteri* so closely matches the peak of the frequency response function $R(\omega)$, and *Chlamydomonas* itself displays a coincidence of its phototactic response and rotation period (34, 35), it is natural to ask whether other species in the same evolutionary lineage, or indeed the larger class of phototactic organisms, can be understood within the present formalism. The allometry of the adaptation time is therefore a key feature for study. It is also of considerable interest to ascertain the
dynamics of chemotaxis in Volvox and to determine its relationship to phototaxis, a linkage proposed for Chlamydomonas (36). Whereas larger multicellular organisms like Volvox can rely on an entirely deterministic mechanism for phototaxis, it remains unclear how stochasticity of motion in unicellular organisms like Chlamydomonas caused by internal biochemical noise (37), affects phototaxis. Finally, the interplay between adaptive flagellar dynamics and the vorticity of natural fluid environments (30) requires further investigation.

Materials and Methods
A detailed description of materials, methods, and supplementary measurements is given in SI Text. A brief summary is given below.

Culture Conditions. V. carteri f. nagaraensis EVE strain was grown axenically in standard Volvox medium (SVM) with sterile air bubbling, in a daily cycle of 16 h of cool white light (4,000 lx) at 28°C and 8 h of darkness at 26°C.

Measuring the Photoreponse to Various Stimuli. Volvox colonies were caught on a rotatable micropipette by gentle aspiration and rotated until the posterior-anterior axis was in the focal plane of the microscope and pointing toward an optical fiber at a distance of ~900 μm. Microscopy was done in red bright-field illumination (λ > 620 nm), to which Volvox is insensitive (15). The flagella-generated flow was visualized with 1-μm polystyrene beads (~1.4 x 10^8 beads per mL in SVM) and recorded at 100 fps. Flow speeds were measured by PIV. The PIV data was interpolated and read out 25 μm above the colony surface—i.e., approximately 10 μm above the flagellar layer. To get a single time series that represents the photoresponse of the colony, we averaged the flow speed time series between ~30° and ~130° as measured from the anterior pole. All stimuli were applied with a cyan LED (500 nm, FWHM 40 nm) coupled into a 550 μm diameter optical fiber. LabVIEW was used to trigger the camera and control the LED light intensity time series. The temperature in the sample chamber was 24.5 ± 0.5°C.

Measuring the Rotation Rate Dependence of the Phototactic Ability. We prepared solutions of SVM with various concentrations of methylcellulose (M0512, Sigma-Aldrich UK), up to 0.65% (wt/wt) (38). From a V. carteri culture that just hatched, phototactic organisms were preselected by a simple test and distributed into rectangular Petri dishes with different concentrations of methylcellulose in SVM. A cyan LED (same as for the optical fiber stimuli) was placed on one side of each Petri dish, providing ~15 μmol photo-synthetically active radiation (PAR) photons μm^-2 s^-1. The Volvox were tracked with software written in Matlab, and rotation frequencies were measured manually. The temperature in the Petri dishes was 24 ± 1°C.

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