NOTE

THE FLAGELLAR PHOTORESPONSE IN *VOLVOX* SPECIES (VOLVOCACEAE, CHLOROPHYCEAE) [1]

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Steering their swimming direction toward the light is crucial for the viability of *Volvox* colonies, the larger members of the volvocine algae. While it is known that this phototactic steering is achieved by a difference in behavior of the flagella on the illuminated and shaded sides, conflicting reports suggest that this asymmetry arises either from a change in beating direction or a change in beating frequency. Here, we report direct observations of the flagellar behavior of various *Volvox* species with different phyletic origin in response to light intensity changes and thereby resolve this controversy: *Volvox barberi* W. Shaw from the section *Volvox* sensu Nozaki (2003) changes the direction of the flagellar beating plane, while species encompassed in the group *Eudorina* (*Volvox carteri* F. Stein, *Volvox aureus* Ehrenb., and *Volvox tertius* Art. Mey.) decrease the flagellar beating frequency, sometimes down to flagellar arrest.

Key index words: motility; multicellularity; phototactic response; turning behavior; Volvocales; *Volvox barberi*

Volvocine algae (Volvocaceae, Chlorophyceae) comprise a monophyletic assemblage of lineages featuring varying degrees of complexity in terms of colony size, colony structure, and cell specialization (Kirk 1998). They range from the unicellular *Chlamydomonas* to colonies made of 4–64 cells with no cellular differentiation (e.g., *Gonium, Pandorina,* and *Eudorina*), to multicellular individuals comprising 1,000–50,000 cells with specialization into reproductive and vegetative functions (*Volvox* sp.) (Koufopanou 1994, Solari et al. 2006a). Volvocine algae have biflagellated cells; colonies with 32 cells or more form spheroids. In the multicellular forms with cellular specialization, each of the *Chlamydomonas*-like somatic cells is positioned at the surface of the extracellular matrix, with its two flagella oriented outward, while the germ cells grow inside the colony. *Volvox* species with germ-soma separation have evolved several times independently from quite different colonial ancestors with no cellular differentiation (Coleman 1999, Nozaki et al. 1999, 2006, Nozaki 2003, Herron and Michod 2008). These species with different phyletic origin have been classified within the volvocine algae into different sections/groups (Smith 1944, Nozaki 2003). Species in different sections/groups differ in characters such as morphology, development, and flagellar structure.

Just as growing toward the light is essential for terrestrial plants and sessile algae, swimming toward the light is essential for motile algae. Unicellular and colonial volvocine algae perform phototaxis, swimming toward or away from light to maximize their photosynthetic rate. Yet, the mechanisms by which motile algae steer toward the light are far from understood. Recent progress has been made on the volvocine algae, where a detailed picture of the photobiochemistry of the eyespot is emerging for *Chlamydomonas* and *Volvox* (reviewed in Hegemann 2008). How this affects the flagellar behavior that eventually leads to phototactic steering remains unclear for *Volvox*, although important progress has been made for *Chlamydomonas* (Kamiya and Witman 1984, Rüffer and Nultsch 1990, 1991, 1995). For *Volvox* species, it is clear that steering toward the light must involve a difference in behavior between the flagella of the somatic cells on the illuminated and shaded sides of the organism. However, it is controversial what constitutes this difference in behavior.

More than a century ago, Holmes (1903) proposed that the somatic cells on the side of *Volvox* that faces the light beat their flagella with less ''vigor'' than those on the shaded side, an idea that has been later termed the ''variable flagellar frequency'' model (Hoops et al. 1999). Like a rowing

boat where the paddles on one side beat with more vigor than those on the other side, this mechanism achieves steering in the correct direction. Several investigators have reported observations that are consistent with this model, including direct observations of flagellar arrest after light stimulation in *V. aureus* (Gerisch 1959, Huth 1970), and observations of the decrease in flagella-generated fluid motion in *V. aureus* (Hand and Haupt 1971, Sakaguchi and Tawada 1977) and *V. carteri* (Sakaguchi and Iwasa 1979). Mast (1926) has proposed a competing model based on observations on an unidentified *Volvox* species that has the same characteristics as the species classified in section *Volvox* sensu Nozaki (2003). In this model, the flagella on the illuminated side of the colony change the beating direction such that they beat more laterally, while those in the shaded side of the sphere beat less laterally and more toward the posterior. Direct observation of flagellar behavior is difficult due to their minute size (∼300 nm diameter) and fast movement (∼25 Hz). This issue caused Hoops et al. (1999) to reinvestigate the behavior of *V. carteri*, finding that the ratio of swimming speed and rotation rate during a phototactic turn supports the variable frequency model. Most recently, Drescher et al. (2010) used high-speed imaging to directly observe the flagellar photoresponse in *V. carteri*. They obtained data consistent with the variable frequency model, which was then used to build a detailed quantitative model of phototactic steering and to explain how the thousands of cells that make up a *Volvox* colony can coordinate their activity through a common photoresponse function and the characteristic spinning of *Volvox* around its axis.

Although most studies provide evidence in favor of the variable frequency model, the compelling and detailed observations on which Mast (1926) based the variable direction model are yet to be explained. Hoops et al. (1999) tried to replicate experiments that were performed with *V. carteri* and *V. aureus* with species from the section *Volvox* sensu Nozaki (2003) (*Volvox rousseletii* and *Volvox capensis*) that had the same characteristics of the unknown species described by Mast (1926), but he was unsuccessful. Here, we propose a resolution to the above controversy by reporting the discovery that *V. barberi*, a species from the section *Volvox* sensu Nozaki (2003), responds to light by changing its flagellar beating direction, while *V. aureus*, *V. tertius*, and *V. carteri* from the group *Eudorina* sensu Nozaki (2003) respond to light by changing the beating frequency.

V. barberi (Carolina Supplies cat# 15-2660, Burlington, NC, USA), *V. rousseletii* (UTEX LB1861, University of Texas at Austin), *V. carteri* f. *nagariensis HK10* (UTEX LB1885), *V. carteri* f. *weismannia* (UTEX LB1876), *V. tertius* (SAG 88-3, Göttingen, Germany), and *V. aureus* (SAG 88-1) populations were cultured in test tubes at low organism concentration (≤10 spheroids · mL^{-1}) in standard *Volvox* medium (Kirk and Kirk 1983), using a daily cycle of 16 h in homogeneous cool-white light (∼40 μmol PAR photons · m^{-2} · s^{-1}) at 28°C, and 8 h of darkness at 26°C. Flagellar behavior was visualized in vivo with an extralong working distance ×40 objective (Nikon, Melville, NY, USA), and recorded with a high-speed camera at 500 frames · s^{-1} (Phantom V5.1; Vision Research, Wayne, NJ, USA), while holding the spheroid in place by micropipette aspiration (Solari et al. 2006b, Drescher et al. 2010). To provide microscope bright-field illumination to which *Volvox* is insensitive (Sakaguchi and Iwasa 1979), an interference filter with transmission $T < 10^{-3}$ for $\lambda < 620$ nm was used (Knight Optical, Harrietsham, UK). A cyan light-emitting diode (M505L1; Thorlabs, Ely, UK), controlled via LabView (National Instruments, Austin, TX, USA), was used to provide a light stimulus (a step increase from 0 to ∼100 μmol PAR photons · m^{-2} · s^{-1}) during the high-speed camera recording. To enhance the visibility of the flagella, the movies were image-processed by subtracting the mean image and by histogram equalization.

High-speed imaging of the photoresponse in *V. barberi* (Fig. 1; two movies are available as supplementary material) clearly shows that the flagella change their beating direction from predominantly toward the posterior to predominantly laterally. By
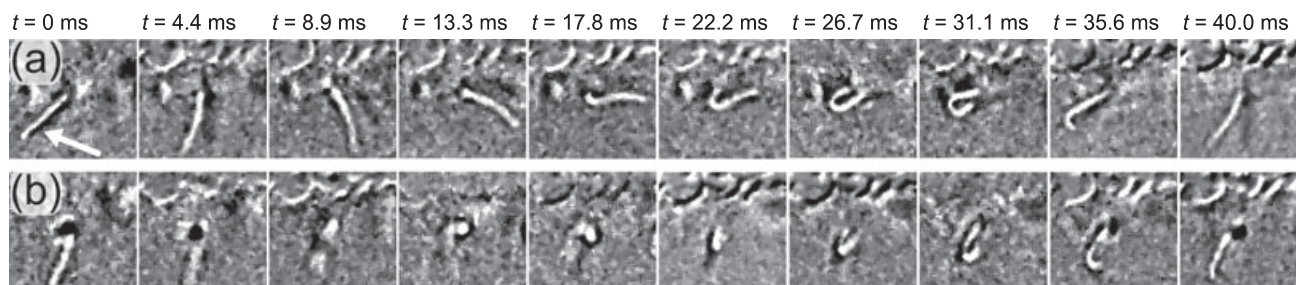

FIG. 1. Light-induced change in flagellar beating direction in *Volvox barberi*. (a) One period of flagellar beating before light stimulation. A white arrow points to the flagellum. The flagellar beating plane is almost exactly within the focal plane. (b) After a step-up in light intensity, the flagellar beating plane is almost perpendicular to the focal plane. Movies are available in the supplementary material.
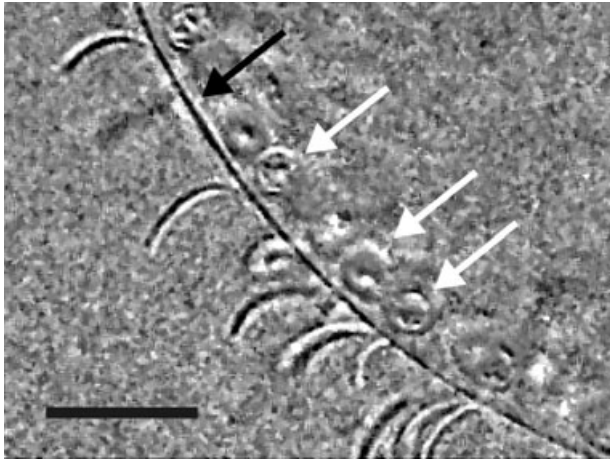
Fig. 2. Flagellar arrest upon a step-up in light intensity, in *Volvox aureus*. The flagella adopt a characteristic, slightly curved shape. White arrows point to somatic cells, while a black arrow points to the boundary of the extracellular matrix. Scale bar, 20 μm.

imaging the flagella on the *Volvox* surface closest to the objective, rather than those on the perimeter as in Figure 1, we could directly observe that the change in beating direction is ∼90°. For *V. carteri* strains, *V. aureus*, and *V. tertius*, we determined that light triggers a decrease in flagellar frequency, which can lead to flagellar arrest in one side of the anterior part of the colony, in a characteristic shape first described by Gerisch (1959) and shown in Figure 2. All responses were transient, lasting only a few seconds (Drescher et al. 2010). Like Hoops et al. (1999), we were unable to observe a photoresponse in *V. rousseletii*; light stimulation did not change flagellar beating frequency or direction in these colonies.

Our observations of the photoresponse of *V. barberi* suggest that Mast (1926) based his hitherto unconfirmed descriptions on this species (or a close relative from section *Volvox*), which resolves the controversy about the *Volvox* phototactic steering response. Further support for this interpretation comes from the unusually high somatic cell count that Mast reported (22,000; see Solari et al. 2008), from the presence of cytoplasmic bridges between the somatic cells, and from the nonspherical somatic cell shape that Mast described—all of which are consistent with *V. barberi*. During the review process for this manuscript, new results have been published (Ueki et al. 2010) that confirm our interpretation. Using a *V. rousseletii* strain that is different from ours, Ueki et al. found that another species from the section *Volvox* displays a change in flagellar beating direction that is similar to the one we describe for *V. barberi*.

In conclusion, we note that the flagellar response to light stimulation of eyespots in the volvocine algae is a fascinating issue with many open questions. Central among them is how the electrophysio-logical signals shape the flagellar beating pattern. Our observations add another facet to this question: How can *V. barberi* change the beating direction of its flagella? Is the internal flagellar structure actuated in a different manner, or do the basal bodies rotate? Differences in the flagellar apparatus between *V. rousseletii* (closely related to *V. barberi*) and *V. carteri* have already been observed (Hoops 1984). Ultrastructural images of the flagellar apparatus in *V. barberi*, and their comparison to species from the other sections, could provide a useful basis for understanding this behavior.

Finally, we want to emphasize that numerous studies have shown that species from the section *Volvox* (e.g., *V. barberi*, *V. rousseletii*, *Volvox globator*) have a distinct phylogenetic origin (Coleman 1999, Nozaki 2003, Herron and Michod 2008) and profound morphological and developmental differences as compared to *Volvox* species in other sections/groups of the phylogenetic tree. We believe that our results further support the need for the creation of a new genus for *Volvox* species in the section *Volvox*.

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Supplementary Material

The following supplementary material is available for this article:

Movie S1. File name "BarberiEquatorSide60x-Filter": Change in flagellar beating direction after light stimulus viewed from the side of a *Volvox barberi* colony. Flagellar behavior was recorded with a high-speed camera at 500 frames per second.

Movie S2. File name "BarberiEquatorCarpet60x": Change in flagellar beating direction after light stimulus viewed from the top of a *Volvox barberi* colony. Flagellar behavior was recorded with a high-speed camera at 500 frames per second.

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