Research Review: Evolution of Biological Complexity

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Abstract

The Volvocine algae lineage, which ranges from single-celled to many-celled organisms are some of the most complex organisms with no cell-cell communication in the many-celled species/genera. These organisms rotate about a fixed body axis and carry out phototaxis in which they swim to optimise light levels - this process is carried out independently in each cell. When misaligned with the light each cell gets an oscillation light signal due to the rotation. Therefore, an adaptive linear model is used to explain phototaxis which fits well across the Volvocine algae: Single-celled Chlamy, 16-celled Gonium and > 1000-celled Volvox. Overall, the shaded side of the organism beats more than the illuminated side, leading orientation towards the light in all Volvocine algae. The timescale of adaption is tuned to the time taken for the organism to complete a rotation.

total words: 2993

1 Introduction

In the summer of 1700, inventor of the microscope, Anthony van Leeuwenhoek, wrote to the Royal Society about his findings in some pond water [Leeuwenhoek, 1700]. He had seen a colony of a few thousand rotating cells (see figure 1) which was to be named *Volvox* [Linne and Salvius, 1758]. In the years since, many more Volvocine algae (VA) have been discovered and classified over many orders of cell number (see figure 2). From the single cell *Chlamydomonas reinhardtii* (Chlamy) to the thousands of cell colonies of *Volvox carteri* (Volvox), in which the cells are attached to an extracellular matrix (ECM). The cell size remains roughly constant throughout the lineage meaning the VA occur at many orders of magnitude in size from $\sim 10\mu m$ to $> 500\mu m$.

VA are photosynthetic organisms, this means light is required to produce nutrients. Phototaxis is the process of photosynthetic organisms moving to achieve optimal light levels. Algae need enough light to produce food but not too much light that it becomes damaging. In this research review only positive phototaxis, in which organisms try to maximise illumination, will be focused on.

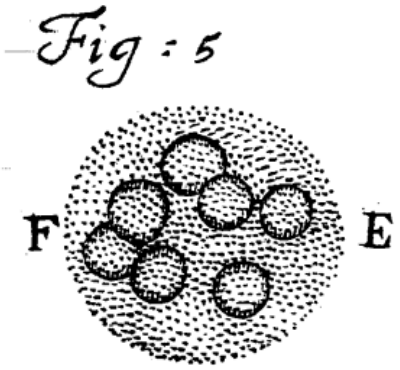

Figure 1: The first ever sketch of a Volvox colony [Leeuwenhoek, 1700]

Multi-cellular VA are some of the most complex organisms that do not have cell-cell communication [HIATT and HAND, 1972], this allows for investigation of how processes such a phototaxis change with size and cell number without other processes (e.g. cell-cell communication) getting in the way. There is thought to have been a Chlamy-like common ancestor to all VA around 200MYA ago [Kirk, 2005]. This is recent when compared to 600 and 450 MYA ago for animals and plants respectively. The speed of evolution suggests that only essential genetic changes were made for multi-cellularity to emerge meaning the underlying biochemistry of the most fundamental processes such a phototaxis would be broadly unchanged.

The basic structure of an individual cell is conserved for both single and many celled species consisting of two flagella, which are microscopic appendages that allow for swimming, and an eyespot which produces a signal in response to changing illumination levels (photoresponse). The eyespot is has a limited 180° of vision due to the cell body blocking the rear 180° and the illumination is related to the projection of the illumination vector onto the eyespot normal vector. Therefore for an angle ψ between the eyespot normal and the illumination vector, the signal received on the eyespot $\propto H(\cos\psi)$, where H is the Heaviside step function.

2 Spinning and Sampling

The two flagella in Chlamy pull the organism forwards by beating breastroke in the $e_2 - e_3$ plane at 45° to the eyespot (see figure 3). When in the steady-state the flagellum nearest the eyespot (cis) is dominant over the other flagellum (trans), sweeping out a greater area in each beat causing a greater torque - this causes rotation about the e_1 axis [Isogai et al., 2000]. There is also a rotation

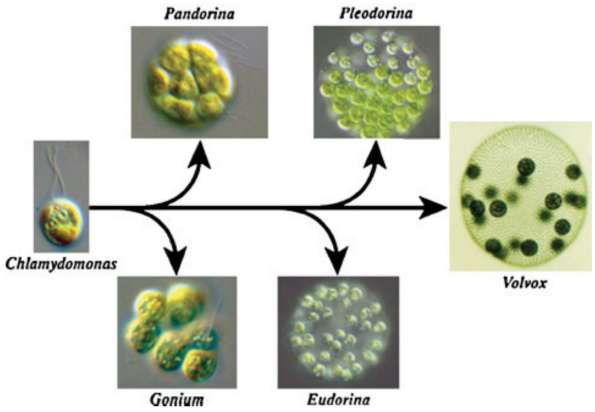

Figure 2: The evolutionary lineage for some of the extant Volvocine algae [Kirk, 2005]

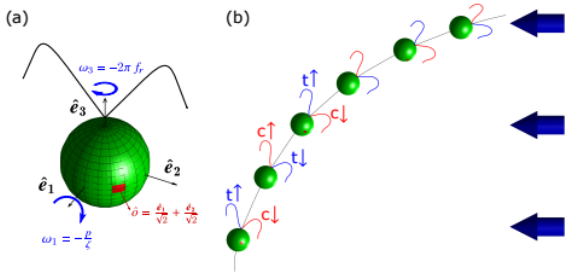

Figure 3: (a) the principle axes of a Chlamy, the flagella mainly beat in $e_2 - e_3$ plane (b) Shows how the behaviour of each flagellum changes to allow for alignment with light source [Leptos et al., 2018]

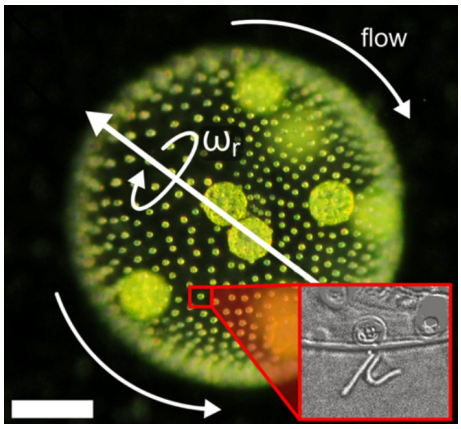

Figure 4: Volvox colony, which rotates about its direction of motion (scale $= 100\mu m$). Zoomed image shows the two flagella of a cell attached to the extra cellular matrix [Drescher et al., 2010]

about about e_3 axis due to the flagella beating slightly out of the $e_2 - e_3$ plane causing an azimuthal torque [Cortese and Wan, 2021]. The combination of these two rotations leads to helical motion of Chlamy in which the eyespot has a constant position with respect to the helix (i.e. it always points outwards) [Crenshaw, 1993]. During the early life of a Volvox colony, the cells are attached to the ESM such that all the flagella beat from the top of the cell towards the bottom during the power stroke. The flagella are also at a slight angle from purely top to bottom as this produces an azimuthal torque which causes the rotation of colony about its direction of travel (see figure 4). The path of a single cell from a Volvox colony therefore travels in a helix (imagine the line plotted by a single Volvox cell over time), the eyespot always faces outwards due to being fixed in place. This shows that even though the colony wide behaviour of Volvox looks very different to Chlamy (no helix vs. helix), for each individual cells the path and geometry is identical.

This movement taken with the directionality of the eyespot leads to oscillating illumination of the eyespot when the cell/colony is not aligned with the light source and constant illumination of the eyespot when it is aligned [Goldstein, 2015]. Phototaxis, therefore, becomes a problem of minimising the oscillating signal on the eyespot(s) of the organism. Reorientation of cells/colonies occurs via the modification of the flagella beating patterns. Though, once orientated towards the light it is most beneficial for the flagella activity to return to normal. This is the motivation for adaptive phototaxis.

The phototaxis in VA is adaptive and can introduced via a familiar analogy. When a new smell appears humans initially smell it very quickly and very strongly. After some time awareness of the new smell decreases until it is no longer noticed and if the smell is removed it would not be realised. Similarly, in adaptive phototaxis, a step-up in illumination causes a modified flagella,

that causes reorientation, response on reaction timescale τ_r that is initially very strong before decaying back to steady-state flagella activity on the adaption timescale τ_a. If the illumination steps back down, there is no modification of flagella beating. In section 4 it is shown that the adaptive phototaxis of Volvox cells leads to flagella on the shaded hemisphere beating strongly and the flagella on the illuminated hemisphere beating very weakly. This beat pattern intuitively leads to a torque and therefore rotation towards the light. The larger VA rotate at a lower frequency, it will be shown later why this requires a larger adaption time.

3 Quantifying phototaxis

In 1903, the phototaxis of *V. carteri* was observed ... 'their movement was at first slow... gradually their path became straighter, the orientation to the light rays more exact and their speed more rapid' [Holmes, 1903]. The viewer was confused as the idea that phototaxis was adaptive was not developed at that point. A quantitative model for this adaption took more than 100 years more to come by. It was initially thought that candidate models must be non-linear as many biological processes are, such as an action potential signal through nerve cells, these models can often be hard to understand and gain an intuition for. In 2007, a linear adaptive model was applied to chemoreception in single flagellum sperm cells to great effect [Friedrich and Jülicher, 2007]. This model, seen in equations 1 and 2, was applied to phototaxis in VA with no modifications.

The model consists of coupled differential equations which relate the illumination on the eyespot (s) to some hidden biochemistry (h) and a photoresponse (p). This made the model measurable as the illumination could be set and the photoresponse measured through the modified flagella activity.

$$\tau_r \dot{p} = (s - h)H(s - h) - p \tag{1}$$

$$\tau_a \dot{h} = s - h \tag{2}$$

In multi-cellular VA the photoresponse in each cell is only due its own eyespot as there is no CNS or communication with other cells. The relationship is also governed by the response time, τ_r, and the adaption time, τ_a. The characteristic times depend on the organism for which is model is being used with. The Heaviside step function is present to ensure that the photoresponse and hidden biochemistry terms only change for a step up in light as this is what is seen qualitatively (i.e humans don't notice a smell being removed once they are used to it). Figure 5 shows the expected response of p and h for different illumination patterns.

The modified flagella beating patterns for a step-up in illumination are not the same for different VA. For example, Chlamy is single-celled meaning the modification is asymmetric as this is the only way to create a reorientation torque. Whereas in Volvox, a single cell is small on the scale of the colony, so

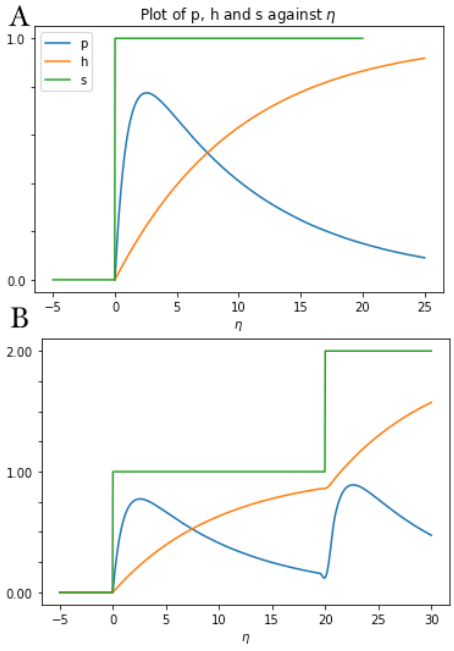

Figure 5: figure shows the computed solutions of 1 and 2 for changing illuminations (a) a single step-up (b) a step-up followed by another step-up. η is used as the non-dimensionalised time variable

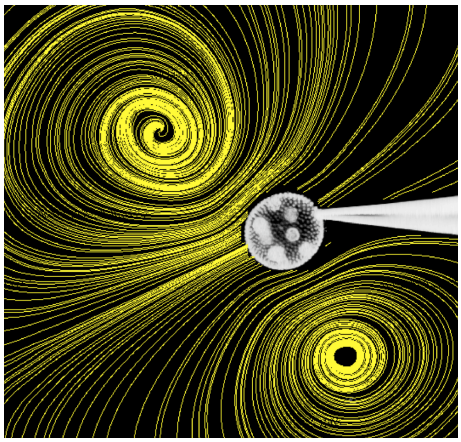

Figure 6: The streamlines of the velocity field found by particle image velocimetry around a Volvox colony localised by a micro-pipette [Goldstein, 2011]

the two flagella are roughly at the same point on the colony so it only makes sense to have a symmetric reaction to the photoresponse. Testing this model for the photoresponse on different VA therefore required different experiments due to the varied ways in which the photoresponse modifies the flagella beating.

4 Testing the theory

4.1 Experimental design

After this new model was suggested for VA, there needed to be a way to test this experimentally. Although in the wild the signal on the eyespot would almost always be oscillating it was desirable to test the photoresponse to a step-up in illumination as it is easiest to test results against the model this way. Producing these results whilst the algae are moving freely would be difficult for two main reasons 1) constant levels of illumination are required on the eyespot (i.e. for step up signals), as VA rotate this would be difficult to achieve 2) the measurable quantities, e.g. flagella activity, would be very difficult to measure to a sufficient resolution when tracking an organism through its swimming. Therefore, in the following experiments VA were localised using a micro-pipette (see figure 6). This allowed for constant illumination on the eyespot from a single light source and for high resolution techniques to be used to quantify the flagella response.

4.2 Photoresponse in Volvox (2010)

In 2010, this model was first tested against Volvox [Drescher et al., 2010]. Single flagella activity was not possible to measure directly due to 3D complexity and small size. The flow around all VA is in the low Reynolds number limit meaning

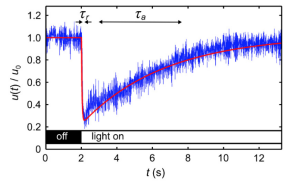

Figure 7: Measuring the linear photoresponse model (equations 1 and 2) indirectly via the velocity just above the flagella as a function of time just after illumination. Data (blue), model from equation 3 (red) [**?**]

viscosity dominates over the inertia of the fluid. Therefore, flagella activity is strongly linked to the velocity field near the flagella as when the flagella stop the fluid stops too due to high viscosity.

$$u = u_0(1 - \beta p(t)) \tag{3}$$

Therefore, a technique called particle image velocimetry (PIV) [Adrian, 2005] can be used to image the streamlines (i.e. the velocity field) of the fluid. This can be done by illuminating and tracking micron sized 'seeding particles' (see figure 6) that are of similar density to the water (e.g. polystyrene balls). The illumination used for tracking is with a wavelength that eyespot is insensitive to.

It was known that, for Volvox, a step-up in illumination decreases both flagella activity and therefore velocity around the colony [Hoops et al., 1999]. Therefore, as the photoresponse increase after a step-up the linear relationship in equation 3 was given between the velocity and photoresponse. Figure 7 shows how the normalised velocity at a single point on a Volvox colony changes after a step-up in illumination. The red line is the model from equation 3 and shows strong agreement with the data.

This data explains what Holmes was seeing in 1903. Once aligned with the light source, the illumination level is constant and high on the top side. Under these constant illumination levels the velocity can recover back to its maximum amount via adaption, this is what was happening when Holmes saw the 'orientation to the light rays more exact and their speed more rapid'.

For a Volvox colony travelling perpendicular to a source of light, cells from the dark side rotate into the light side and the eyespot is illuminated which the down-regulates the flagella on timescale τ_r. The adaption timescale is similar to the time taken for the colony to complete a rotation, so by the time these down regulated flagella have rotated back into the dark side they are beating at near full power. The macro view of this is that you get the shaded hemisphere beating strongly and the illuminated hemisphere beating very weakly. This gives a torque towards the light.

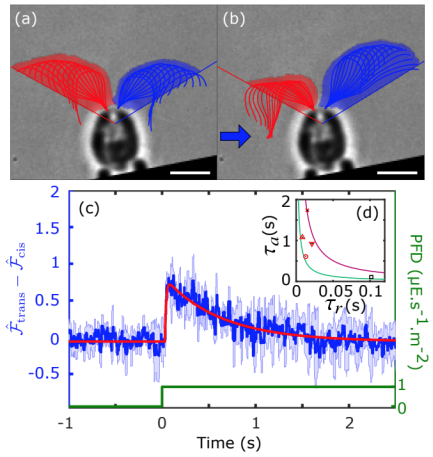

Figure 8: Chlamy localised using micropipette a) area swept out by flagella in steady-state b) area swept out by flagella after illumination c) difference in area swept out by flagella as a function of time d) $\tau_a - \tau_r$ graph [Leptos et al., 2018]

4.3 Photoresponse in Chlamy (2018)

In 2018, the photoresponse in Chlamy was investigated [Leptos et al., 2018]. The organism was again localised using a micropipette. Earlier, it was noted that the flagella of must be asymmetric to produce a torque. Therefore, the area swept out by the cis and trans flagella was a quantity of interest.

In the steady-state figure 8a shows that the cis flagella (red) sweeps a greater area than the trans flagella (blue) causing the rotation about e_1 axis. As expected the flagella respond different to a step-up in illumination the, cis flagella strongly decreased in area and the trans flagella slightly increased in area (see figure **??**b). The difference in the areas swept out by the flagella was measured and this was modelled against the photoresponse. As seen in figure 8c the model (red) matches the data very well. The adaption time for the photoresponse in Chlamy is, like Volvox, roughly the time period for cell body rotation.

As the eyespot rotates from the dark side into the light side the step-up causes the trans flagellum to become dominant. The adaption time for the photoresponse in Chlamy is, like Volvox, roughly the time period for cell body rotation [Yoshimura and Kamiya, 2001]. This means that once the eyespot is facing away from the light, the photoresponse recovers to give cis flagellum dominance. Overall, the flagellum that is on the shaded side will always be dominant causing a turning torque, this can be seen clearly in figure 3b where the flagellum away from the light is always dominant

It is seen that despite Chlamy being single celled and Volvox having 1000s of cells, The same phototaxis is produced with the same photoresponse in each cell. The difference is that the two regimes in Chlamy are two flagella vs. the two hemispheres in Volvox.

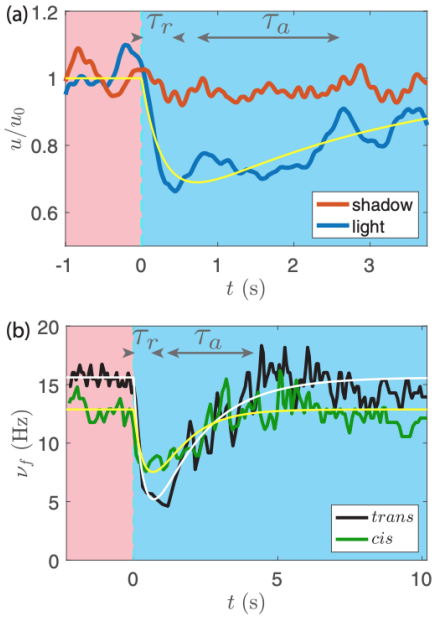

Figure 9: 2020 experiment on Gonium (a) azimuthal measure velocities on the shaded (red), light (blue) and model (equation 3) (yellow) (b) the frequencies of the cis and trans flagella of the peripheral *V. carteri*-like (seen in figure **??** [De Maleprade et al., 2020]

4.4 Photoresponse in Gonium (2020)

In 2020, a similar experiment was again carried out for another VA [De Maleprade et al., 2020]. *Gonium pectorale* (Gonium) is the simplest differentiated colonial algae, meaning multiple types of cells with different functions. Gonium has 16 cells arranged in a disc structure (see figure **??**) with 4 central Chlamy-like cells that pull the disc forwards in a breaststroke and 12 Volvox-like cells on the periphery which beat at $\sim 15^\circ$ to the disc plane providing an azimuthal torque whilst also propelling the organism forwards.

Gonium colonies travel in left-handed helices. This is due to unbalanced flagella forces of peripheral cells causing rotation about e_1. Here, we can cell dominance instead of cis flagellum dominance.

To motivate phototaxis in Gonium only the photoresponse of the peripheral cells needed to be considered. Both flagella down-regulate upon a step-up in illumination, like Volvox, as both flagella have roughly the same position on the scale of the cell. Again via localisation and PIV the data in figure 9 was gathered and the. Figure 9a shows that the photoresponse is used by the peripheral cells in a similar way to Volvox with the down regulation and recovery of the azimuthal velocity (note similarities to figure 7).

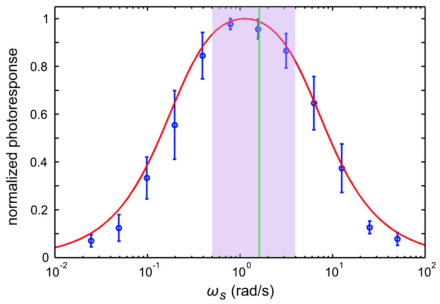

Figure 10: Normalised frequency space photoresponse model from equation 5 (red) and data from *V. carteri* (blue) resembles a band-pass filter [Drescher et al., 2010]

The frequency of the cis/trans flagella of peripheral cells were found (figure 9b) by counting oscillations as number of times through the equilibrium position. Flagella that beat quicker provide a larger force. Figure 9b shows use that without illumination the trans flagella is dominant, beating at a higher frequency. Unlike Chlamy the trans flagella is dominant and not the cis flagella, this may be because in Chlamy flagella pull and in Gonium the peripheral flagella are propelling. Although, upon a step-up illumination both flagella decrease in frequency in Gonium, the decrease of the dominant flagella is most severe, this is the same in Chlamy where the dominant flagella is most affected. This pattern also follows the photoresponse function.

It should also be noted that the adaption time in Gonium is, like Chlamy and Volvox, similar to the rotation time period of the disc. This means that when travelling perpendicular to the light, the shaded edge of the disc beats strongly and the illuminated edge beats very weakly for the reasons given at the end of sections 4.2 and 4.3.

5 Adaptive phototaxis a a band-pass filter

$$p(t) = \frac{s_2 - s_1}{1 - \tau_a/\tau_r}(e^{-t/\tau_a} - e^{-t/\tau_r}) \tag{4}$$

$$R(\omega_s) = \left|\frac{\tilde{p}}{\tilde{s}}\right| = \frac{\omega_s \tau_a}{\sqrt{(1+\omega_s^2\tau_r^2)(1+\omega_s^2\tau_a^2)}} \tag{5}$$

During the experiment that first tested the photoresponse function against Volvox [Drescher et al., 2010], some measurements were taken with the illumination being turned on and off at different frequencies. This allowed for the frequency space photoresponse of cells to be recorded. Analytically, the solution to the differential equations 1 and 2 gives 4, taking the Fourier transform of this gives 5 - this is the response function.

The data for Volvox fitted this analytical solution to the model well (red line), the frequency space photoresponse resembled a band-pass filter centred on the mean frequency of cell body rotation (vertical green line). As discussed, when swimming misaligned to the illumination there will be an oscillating signal on each eyespot at the frequency of cell body rotation. Oscillating signals at this frequency maximise the photoresponse as seen by the position of the green line in figure 10. Frequencies that are not of order cell body rotation are heavily suppressed. For example, very rapid frequencies (such a shimmer from the water surface) or very slow frequencies (such as clouds slowly passing). This leads to distraction-free phototaxis [Goldstein, 2011]. This theory was further proved when younger Volvox colonies which are smaller and rotate quicker, with a period more closely matching τ_a, are seen to carry out phototaxis more effectively than older slower rotating colonies.

6 Discussion

VA are a unique opportunity to study the evolution of complexity over many cell numbers without any cell-cell communication. Phototaxis in these rotating algae is a problem of reducing the oscillating light signal on the eyespot which occurs, in both single and multi-cellular species/genera. In control theory terms, VA are well adapted as it is far easier to precisely remove an oscillating signal than make a stationary one constant. When misaligned the response to turn towards the light is the same for all genera, with the shaded side having more activity than the illuminated one. Though, due to the differing symmetries, each side manifests itself differently - a single flagellum in Chlamy, an edge of the disc in Gonium and a hemisphere in Volvox (see figure **??**). Therefore, in multi-cellular VA, despite the photoresponse in each cell being independent from any other cell, there is a unified response to turn towards the light. Investigation about how these ideas could be used to more effectively decentralise complex engineering problems - this can decrease failure rate when a single component of a system breaks.

Rotation speed of VA decreases as the cell number increases. This is important as phototaxis is adaptive - the modified flagella response adapts back to its steady-state on some characteristic timescale. Experiments have shown that the photoresponse in each species/genera is tuned so that the adaption timescale is similar to the time for a rotation (i.e. $f_{rot}\tau_a \sim 1$). Therefore, the photoresponse of each cell acts as a band-pass filter, centred on the frequency of cell body rotation, ensuring only oscillations due to the rotation are 'listened' to.

Biologically it is of interest to know how the same photoresponse function that caused asymmetric modified flagella beating in a Chlamy-like ancestor could internally 'rewire' to give a symmetric response in Volvox today. This could be investigate by researching intermediates, like with Gonium in section 4.4, by seeing what asymmetric and symmetric features are present in the modified flagella response.

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