Flagellar phenotypic plasticity in volvocalean algae correlates with Péclet number

Cristian A. Solari1, Knut Drescher2, Sujoy Ganguly2, John O. Kessler3, Richard E. Michod4 and Raymond E. Goldstein2,*

1 CONICET, Laboratorio de Biología Comparada de Protistas, Departamento de Biodiversidad y Biología Experimental (FCEN), Universidad de Buenos Aires, C1428EHA Buenos Aires, Argentina
2 Department of Applied Mathematics and Theoretical Physics, Centre for Mathematical Sciences, University of Cambridge, Wilberforce Road, Cambridge CB3 0WA, UK
3 Department of Physics, and 4 Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA

Flagella-generated fluid stirring has been suggested to enhance nutrient uptake for sufficiently large micro-organisms, and to have played a role in evolutionary transitions to multicellularity. A corollary to this predicted size-dependent benefit is a propensity for phenotypic plasticity in the flow-generating mechanism to appear in large species under nutrient deprivation. We examined four species of volvocalean algae whose radii and flow speeds differ greatly, with Péclet numbers (Pe) separated by several orders of magnitude. Populations of unicellular Chlamydomonas reinhardtii and one- to eight-celled Gonium pectorale (Pe ≈ 0.1–1) and multicellular Volvox carteri and Volvox barberi (Pe ≈ 100) were grown in diluted and undiluted media. For C. reinhardtii and G. pectorale, decreasing the nutrient concentration resulted in smaller cells, but had no effect on flagellar length and propulsion force. In contrast, these conditions induced Volvox colonies to grow larger and increase their flagellar length, separating the somatic cells further. Detailed studies on V. carteri found that the opposing effects of increasing beating force and flagellar spacing balance, so the fluid speed across the colony surface remains unchanged between nutrient conditions. These results lend further support to the hypothesized link between the Péclet number, nutrient uptake and the evolution of biological complexity in the Volvocales.

Keywords: phenotypic plasticity; evolution; Volvox; flagella; fluid dynamics; nutrient uptake

1. INTRODUCTION

A fundamental subject in evolutionary biology is the evolutionary transition from unicellular organisms to multicellular ones, and the accompanying cellular differentiation and specialization [1,2]. Not surprisingly for micro-organisms living in an aqueous environment, many of the important factors are physical, involving diffusion and mixing, for the exchange of molecular species with the environment is one of the most basic factors of life. Progress on this important evolutionary issue therefore involves not only studies of molecular and genetic aspects of multicellular life, but also the introduction of techniques from transport theory to address the allometric scaling of metabolic activity with size [3].

At least since the work of Weismann [4], it has been recognized that a particularly interesting class of organisms to study for insights into the origins of multicellularity is composed of the alga Volvox and its relatives. Volvocalean green algae are motile micro-organisms consisting of biflagellated cells. They range from the unicellular Chlamydomonas to colonies made of cells with no cellular differentiation, such as Gonium (one to eight cells), Eudorina (4–64 cells) and Pleodorina (16–256 cells), to the multicellular Volvox comprising 500–50 000 cells with specialization in reproductive and vegetative functions, i.e. germ–soma separation (figure 1; [6–8]). In the multicellular forms, each of the Chlamydomonas-like somatic cells is found at the surface of the extracellular matrix (ECM), with its two flagella oriented outwards, while the germ cells lose their flagella and grow on the inside of the colony (figure 1). The somatic cells may be connected through cytoplasmic bridges, as in Volvox barberi, or unconnected, as in Volvox carteri. Germ–soma separation in Volvox species such as V. carteri and V. barberi has evolved independently

*Author for correspondence (r.e.goldstein@damtp.cam.ac.uk).
from different ancestors [9–13]. In short, Volvocales comprise a group of closely related lineages with different degrees of cell specialization which seem to represent ‘alternative stable states’ [14] that reflect clearly the stages of the transition to multicellularity and cellular differentiation.

Volvocales are found in quiet, standing waters of transient vernal puddles or in permanent lakes where thermal stirring stops and the lake becomes stratified [6,15]. Because they are negatively buoyant, these organisms need flagellar beating to avoid sinking and to reach light and nutrients [7,8]. In addition to providing motility, flagella may also be important for generating advective flows that can increase nutrient uptake. If the Volvocales were to rely on diffusion alone to acquire nutrients from a quiescent fluid environment, the total rate of uptake would scale linearly with organism size. In contrast, the metabolic needs of Volvocales that form spheroids grow at least quadratically [5], implying that there is a bottleneck that the demands on the flagella change with organism size. The emerging hypothesis is therefore that, for larger Volvocales, the collective beating of flagella not only yields motility, but also improves the molecular transport of nutrients, waste products and chemical messengers.

To quantify this hypothesis, we note that the Volvocales, along with most other micro-organisms, live in a world of Reynolds number \(Re \ll 1\) [18,19]. In this ‘Stokes flow’ regime, motion is dominated by viscosity, fluid flows are linear and time reversible and nutrient transport is usually dominated by diffusion. However, on the surface of a Volvox colony, the collective beating of many closely spaced flagella can lead to fluid flows of sufficiently high speeds that nutrient transport by advection may replace diffusion as the most important mechanism. The relative importance of these transport processes can be quantified by first defining a typical fluid velocity \(U\), length scale \(L\) and diffusion constant \(D\) \((D \sim 2 \times 10^{-5} \text{ cm}^2 \text{s}^{-1} \text{ for } \text{O}_2 \text{ is typical for small molecules})\). Then, a dimensionless ratio of the time scale for diffusion \((t_{\text{diff}} = L^2/D)\) and advection \((t_{\text{adv}} = L/U)\), known as the Péclét number \((\text{Pe} = t_{\text{diff}}/t_{\text{adv}} = UL/D)\), serves to characterize the relative importance of the two processes. If \(\text{Pe} \ll 1\), diffusion is faster than the transport of molecules by advection via the flowing medium, indicating that an organism does not need to invest in flagellar beating to increase nutrient uptake. If however \(\text{Pe} \gg 1\), advection through collectively generated flows may be important. For Volvox colonies, the flagellar beating leads to \(\text{Pe} \gg 1\), while for the unicellular \textit{Chlamydomonas} \(\text{Pe} \sim 0.1\) [16]. Self-generated flows (figure 2), produced by hundreds or thousands of somatic cells arrayed on the surface of Volvox, may thus free these large spherical colonies from the constraints of diffusion-limited nutrient uptake, facilitating the transition to multicellularity and germ–soma differentiation.

If the larger Volvocales have come to depend upon fluid flow generated by beating flagella for enhanced nutrient uptake, it stands to reason that conditions of nutrient deprivation might trigger changes in the motility apparatus to mitigate such an environmental stress. On the other hand, for much smaller organisms like \textit{Chlamydomonas} and \textit{Gonium}, such effects would not be expected. This type of response would be an example of phenotypic plasticity, defined as the production of multiple phenotypes from a single genotype, depending...
Volvocalean algae: phenotypic plasticity C. A. Solari et al.

2. MATERIAL AND METHODS

Populations of *V. carteri* f. *nagariensis* EVE strain (kindly provided by D. L. Kirk), *V. barberi* (Carolina Supplies, cat. no. 152660), *C. reinhardtii* (UTEX 89) and *G. pectorale* (UTEX LB826) were synchronized in test tubes with 20 ml of standard *V. carteri* medium (SVM; [22]), and illuminated by homogeneous cool white light [approx. 1000 foot candles; fc (1fc = 10.764 lux)] in a daily cycle of 16 h of light (at 28°C) and 8 h of darkness (at 26°C). Under these conditions, the asexual life cycle of *C. reinhardtii* and *G. pectorale* has a 24 h generation time; cells grow during the light period, perform multiple divisions in the dark and daughter cells and colonies (for *Gonium*) are released when light returns. The asexual life cycle of *V. barberi* and *V. carteri* takes 48 h under these conditions, and is shown for *V. carteri* in figure 3.

To check for phenotypic plasticity, individuals were grown in two different nutrient concentrations as follows. From a synchronized population, just after individuals hatched (3, 6, 2 and 2 h into the light period for *V. carteri*, *V. barberi*, *C. reinhardtii* and *G. pectorale*, respectively), individuals were harvested by slow centrifugation, transferred to distilled water, centrifuged again and randomly separated into two sub-populations: one placed in full-strength SVM and the other in a 10⁻³ dilution of SVM. For each species and for both nutrient treatments, the organism concentration was adjusted to approximately 10⁴ cells ml⁻¹. The organism concentrations were therefore 10⁴ *C. reinhardtii* cells ml⁻¹, approximately 1400 *G. pectorale* colonies ml⁻¹ (colonies had on average seven cells), five *V. carteri* spheroids ml⁻¹ (organisms had on average 2000 cells) and two *V. barberi* spheroids ml⁻¹ (organisms had on average 5000 cells). Measurements were performed after the organisms were grown in the diluted and undiluted SVM for 8 h. During these 8 h, and the ensuing measurement period, all species were in their growth phase. During the experiments, cellular division did not take place in any species, except in *V. barberi*. In contrast to the other species, in which the reproductive cells grow about 2⁺fold in size and then undergo a rapid series of n synchronous divisions (the ‘palintomic’ ancestral developmental programme), the reproductive cells of *V. barberi* have a derived developmental programme and perform binary fission to produce daughter colonies [13,23,24].

In all experiments, digital images were taken at high magnification and analysed with image processing software (METAMORPH, Universal Imaging Corp., PA, USA) to measure flagellar lengths, diameters of cells and *V. carteri* spheroids and the number and diameter of reproductive and somatic cells. Cell and spheroid diameters were measured by taking the mean of two orthogonal diameters. The number of somatic cells per unit area on the surface of a *V. carteri* spheroid was calculated by taking the mean somatic cell concentration from two opposite sides of the spheroid. A multiple linear regression (MLR) analysis was performed (JMP software; SAS Institute, Cary, NC, USA) using indicator variables to account for the nominal factors. Continuous variables were used to account for factors such as flagellar length and colony cell number.

2.1. Initial experiments on the phenotypic plasticity of the organism and flagellar sizes

Measurements were performed at two times (t₁ and t₂) in the life cycle of the organisms: t₁ = just before the organisms were harvested (as detailed above), and t₂ = 8 h later on the same day. For these measurements, Lugol solution was used to fix 1 ml samples of all organisms, except *V. barberi*. Samples of *V. barberi* were fixed
with formalin instead of Lugol solution because the Lugol solution made their flagellar curl. Spheroid size, cell size and flagellar length were measured for 10 individuals for each nutrient treatment and species. For *G. pectorale*, measurements were averaged from two cells in each colony; for *Volvox* species, measurements were averaged on two germ cells and five somatic cells in each colony. The experiments were repeated three times, yielding for each species data on \( n = 30 \) organisms at \( t_1 \) and \( n = 60 \) organisms between the two nutrient treatments at \( t_2 \).

### 2.2. Experiments for propulsion force measurement of *C. reinhardtii* and *G. pectorale*

To check for differences in propulsion force between the nutrient treatments, upward swimming \( V_{up} \) and sedimentation \( V_{sed} \) speeds were measured as detailed in Solari *et al.* [8] using the apparatus developed by Drescher *et al.* [25]. The growth conditions were as described in §2.1, but with a lower light intensity (approx. 600 fc) as the cultures for these experiments were grown in a different country and a different diurnal chamber that did not allow a higher light intensity. For each experiment, we measured \( V_{up} \) and \( V_{sed} \) of 30 individuals and the radius \( R \) of 15 individuals. As described in Solari *et al.* [8], the propulsion force \( F_p \) exerted by an individual swimming vertically upward at velocity \( V_{up} \) is balanced by the sum of the drag force and gravity, \( F_p = 6\pi\eta R (V_{up} + V_{sed}) \). By using the population average of \( V_{up} \), \( V_{sed} \) and \( R \) in this equation, we obtain the population average of \( F_p \) for the two treatments. The experiments were repeated four times, yielding for each species data on \( n = 8 \) populations between the two nutrient treatments at \( t_2 \).

### 2.3. Detailed experiments on the phenotypic plasticity of *V. carteri*

Flagellar lengths, beating frequencies and flagella-driven flow speeds of *V. carteri* were measured *in vivo*, while holding the spheroid in place by micropipette aspiration [16], as shown in figure 2. Spheroid sizes, cell sizes and flagellar lengths were measured as described in §2.1, but only at \( t_2 \). The growth conditions were as described in §2.1, but with a lower light intensity (approx. 600 fc) as in §2.2. Measurements were performed in the 2 h period beginning at \( t_2 \). This period was further divided into four sub periods of 30 min; in each sub period, measurements on five spheroids from the same nutrient treatment were performed, and the sub periods of measurements from each treatment were alternated (e.g. A/B/A/B or B/A/B/A). Flagella-driven flows around *V. carteri* were visualized with 1 \( \mu \)m micro-spheres (Invitrogen Corp., CA, USA), recorded with a high-speed camera (Phantom v. 5.1, Vision Research, NJ, USA) and measured using particle image velocimetry (FlowManager, Dantec Dynamics, Skovlunde, Denmark). For the

---

*J. R. Soc. Interface* (2011)
Table 1. Data from populations grown at a light intensity of 1000 fc, i.e. experiment described in §2.1, in the format average ± s.e. The number of organisms n that make up an average value is n = 30 in each case. The times t1 and t2 at which measurements were conducted are 1–2 h after hatching and 8 h later, respectively. The difference between treatments is given in absolute terms as the difference between the measurements in normal and diluted media at t2, as obtained with an MLR model. The statistical p-value was obtained from the MLR model. A * marks statistically non-significant differences between treatments. Details of the MLR model used here are given in Table 3. The symbols used are cell radius rC, and flagellar length l, and Volvox spheroid radius R, germ cell radius rG, somatic cell radius rS and somatic cell concentration C. The average cell number of a G. pectorale colony was 7.0 ± 0.28. The average total number of somatic cells of a V. carteri colony was 1970 ± 56, and the average total number of germ cells was 11.9 ± 0.2. The average total number of somatic cells of a V. barberi colony was 4975 ± 336, and the average number of germ cells was 19.9 ± 0.5.

<table>
<thead>
<tr>
<th></th>
<th>t1: normal medium</th>
<th>t2: normal medium</th>
<th>t2: diluted medium</th>
<th>absolute</th>
<th>(%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. reinhardtii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rC (µm)</td>
<td>3.43 ± 0.07</td>
<td>5.24 ± 0.20</td>
<td>4.73 ± 0.16</td>
<td>−0.36 ± 0.18</td>
<td>−6.9 ± 3.4</td>
<td>0.0431</td>
</tr>
<tr>
<td>l (µm)</td>
<td>10.4 ± 0.33</td>
<td>9.07 ± 0.40</td>
<td>9.11 ± 0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. pectorale</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rC (µm)</td>
<td>4.66 ± 0.11</td>
<td>5.51 ± 0.15</td>
<td>5.03 ± 0.16</td>
<td>−0.34 ± 0.16</td>
<td>−6.2 ± 2.9</td>
<td>0.0204</td>
</tr>
<tr>
<td>l (µm)</td>
<td>17.17 ± 1.29</td>
<td>17.75 ± 0.67</td>
<td>19.06 ± 0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. carteri</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R (µm)</td>
<td>155 ± 3.9</td>
<td>204 ± 3.9</td>
<td>222 ± 4.0</td>
<td>14.6 ± 4.86</td>
<td>7.2 ± 2.4</td>
<td>0.0031</td>
</tr>
<tr>
<td>rG (µm)</td>
<td>24.1 ± 0.76</td>
<td>30.1 ± 0.40</td>
<td>29.3 ± 0.34</td>
<td>−1.16 ± 0.63</td>
<td>−3.9 ± 2.1</td>
<td>0.0691</td>
</tr>
<tr>
<td>rS (µm)</td>
<td>4.04 ± 0.09</td>
<td>4.89 ± 0.09</td>
<td>4.37 ± 0.07</td>
<td>−0.46 ± 0.09</td>
<td>−9.4 ± 1.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>l (µm)</td>
<td>14.9 ± 0.71</td>
<td>18.2 ± 0.50</td>
<td>20.45 ± 0.46</td>
<td>2.14 ± 0.68</td>
<td>11.7 ± 3.7</td>
<td>0.0021</td>
</tr>
<tr>
<td>C (cells/10^4 µm^2)</td>
<td>6.91 ± 0.49</td>
<td>3.68 ± 0.29</td>
<td>3.17 ± 0.18</td>
<td>−0.65 ± 0.33</td>
<td>−17.7 ± 9.1</td>
<td>0.0552</td>
</tr>
<tr>
<td>V. barberi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R (µm)</td>
<td>217 ± 5.4</td>
<td>279 ± 12.4</td>
<td>326 ± 7.2</td>
<td>59.1 ± 11.0</td>
<td>21.1 ± 3.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>rG (µm)</td>
<td>14.0 ± 1.17</td>
<td>27.3 ± 1.21</td>
<td>29.7 ± 1.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rS (µm)</td>
<td>5.19 ± 0.22</td>
<td>7.02 ± 0.17</td>
<td>6.86 ± 0.10</td>
<td>−0.77 ± 0.41</td>
<td>−11.0 ± 5.8</td>
<td>0.0712</td>
</tr>
<tr>
<td>l (µm)</td>
<td>22.9 ± 0.54</td>
<td>28.6 ± 1.96</td>
<td>39.0 ± 2.17</td>
<td>9.93 ± 2.56</td>
<td>34.7 ± 9.0</td>
<td>0.0006</td>
</tr>
<tr>
<td>C (cells/10^4 µm^2)</td>
<td>9.08 ± 1.01</td>
<td>5.42 ± 0.31</td>
<td>3.22 ± 0.31</td>
<td>−1.20 ± 0.57</td>
<td>−22.1 ± 10.5</td>
<td>0.0341</td>
</tr>
</tbody>
</table>

measures of the flow speed, a V. carteri spheroid was caught such that the micropipette aspiration point was approximately on the equator. The micropipette was then rotated until the Volvox anterior–posterior axis was in the focal plane. The flow speed was read out at the Volvox equator on the side opposite the aspiration point, just above the spheroid surface (10 µm above the flagellar tips), as the flow speed reaches a maximum there. This maximum speed U can be related mathematically to the force the flagella generate [5]. Flagellar beating frequencies were determined by averaging across 10 beating periods, and averaging across five somatic cells around the Volvox equator. The experiment was repeated four times, yielding data on n = 80 V. carteri colonies between the two nutrient treatments.

For each Volvox colony, the measured peak fluid speed U was used to estimate the total force F that all flagella exert on the fluid. Using a mathematical model, Short et al. [5] found that \( F = 6\pi \eta RU/3 \), where R is the Volvox radius and \( \eta \) is the viscosity of water. Taking into account that the flagellar force is applied to the fluid from the surface of a sphere, the net forward thrust can be shown to be \( F_p = \pi F/4 \) [26]. The measured colony, somatic and germ cell radii, and the calculated \( F_p \) were then used to estimate the upward swimming speed \( V_{up} \) for each colony. As described in detail in Solari et al. [8], \( V_{up} = (F_p - g\Delta M)/6\pi \eta R \), where g is the acceleration of gravity and \( \Delta M \) is the difference in mass between the cells and the displaced water, assuming that the ECM is approximately neutrally buoyant (measured cell densities were taken from [27]).

3. RESULTS AND DISCUSSION

Table 1 gives results from the initial experiments on phenotypic differences in populations that were grown in normal and diluted media, at a light intensity of 1000 fc. Table 2 contains the results from the more detailed experiments on V. carteri, in which populations were grown at 600 fc. It also contains the swimming and sedimentation speeds, as well as the thrust force calculations of C. reinhardtii and G. pectorale. Figure 4 illustrates the effects of the nutrient deprivation upon nutrient deprivation is not surprising, as nutrient uptake in these organisms is dominated by diffusion (the relevant Péclet number is \( Pe \sim 0.1 \)), implying that C. reinhardtii and G. pectorale can take no measures to oppose starvation if the growth medium is low in nutrients. The swimming and sedimentation speeds and swimming force calculations confirm these
longer flagella and larger spheroids for both species, which contribute to higher swimming speeds. As a result, in the more detailed experiments colonies were grown with lower light intensity (approx. 600 fc instead of approx. 1000 fc), they had fewer cells, and reached a smaller spheroid, cell and flagellar size than in the initial experiments. However, the data from the more detailed experiments had a better statistical significance and qualitatively confirm the results from the initial experiments (see comparison in figure 4). It is worth noting that these experiments showed that regardless of nutrient treatment, colonies with longer flagella have a lower beating frequency within a low-nutrient medium. Regardless of treatment, *V. barberi* has a higher cell concentration per unit area and longer flagella than *V. carteri* (table 1). The initial experiments (table 1) showed that colonies grown in diluted media had smaller somatic cells with longer flagella and larger spheroids for both species, the latter owing to an increased amount of ECM. We investigated in more detail the phenotypic alterations of *V. carteri* (table 2), by using equipment that allowed measurements of the peak fluid speed $U$, the flagellar beating frequency $f$ and the force exerted by the flagella on the fluid $F$. Because in these more detailed experiments colonies were grown with lower light intensity (approx. 600 fc instead of approx. 1000 fc), they had fewer cells, and reached a smaller spheroid, cell and flagellar size than in the initial experiments. However, the data from the more detailed experiments had a better statistical significance and qualitatively confirm the results from the initial experiments (see comparison in figure 4). It is worth noting that these experiments showed that, regardless of nutrient treatment, colonies with longer flagella have a lower beating frequency (table 4). Further, results from these more detailed experiments showed that there was no difference in fluid speed $U$ between treatments, even though the biflagellated somatic cells were more sparsely spaced in the diluted medium owing to the larger spheroid size under those conditions. As there is only very weak evidence for a small increase in flagellar beating frequency upon dilution (table 2), the fact that the flow speed $U$ remains constant despite

Table 2. Data from *C. reinhardtii*, *G. pectorale* and *V. carteri* populations grown at a light intensity of 600 fc, i.e. experiments described in §§2.2 and 2.3. The format and notation are as in table 1. Additional symbols are the flagellar beating frequency $f$, the peak flow speed at the equator $U$ (described in §2), the net force that all flagella exert on the fluid $F$, the propulsion force $F_p$ and the sedimentation speed $V_{sed}$. For each measurement on *V. carteri*, $n = 40$ colonies were used. For measurements on *C. reinhardtii* and *G. pectorale*, $n = 120$ individuals were used for $V_{up}$ and $V_{sed}$ and $n = 4$ populations for $F_p$. Details of the MLR model used here are given in table 4. The average number of somatic cells of a *V. carteri* colony was 1557 ± 50, and the average number of germ cells was 10.0 ± 0.2. In these experiments, the average number of cells in *G. pectorale* colonies was 4.1 ± 0.19.

<table>
<thead>
<tr>
<th>Species</th>
<th>$t_2$: normal medium</th>
<th>$t_2$: diluted medium</th>
<th>absolute difference</th>
<th>(% difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{sed}$ ($\mu$m s$^{-1}$)</td>
<td>6.5 ± 0.24</td>
<td>3.7 ± 0.14</td>
<td>−2.75 ± 0.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$V_{up}$ ($\mu$m s$^{-1}$)</td>
<td>43 ± 1.9</td>
<td>54 ± 2.6</td>
<td>11.41 ± 3.32</td>
<td>0.0007</td>
</tr>
<tr>
<td>$F_p$ (pN)</td>
<td>5.59 ± 0.57</td>
<td>5.13 ± 0.39</td>
<td>$\frac{\bar{y}}{\bar{y}}$</td>
<td>0.5367</td>
</tr>
<tr>
<td>$V_{sed}$ ($\mu$m s$^{-1}$)</td>
<td>10.0 ± 0.39</td>
<td>8.9 ± 0.37</td>
<td>−1.11 ± 0.54</td>
<td>0.0490</td>
</tr>
<tr>
<td>$V_{up}$ ($\mu$m s$^{-1}$)</td>
<td>33 ± 1.3</td>
<td>37 ± 1.1</td>
<td>4.21 ± 1.76</td>
<td>0.0176</td>
</tr>
<tr>
<td>$F_p$ (pN)</td>
<td>8.89 ± 0.93</td>
<td>9.06 ± 0.60</td>
<td>$\frac{\bar{y}}{\bar{y}}$</td>
<td>0.8847</td>
</tr>
</tbody>
</table>

Figure 4. Bar chart showing the percentage changes of properties of *V. carteri* colonies grown in diluted medium, with respect to those grown in normal medium. Two colours indicate results from the different experiments: table 1 in blue (1000 fc), and table 2 in red (600 fc). Error bars show the standard error. The symbols used are the spheroid radius $R$, somatic cell radius $r_s$, flagellar length $l$, somatic cell concentration $C$, flagellar frequency $f$, net force of flagella on the fluid $F$, and the upward swimming speed $V_{up}$. Results on *C. reinhardtii* and *G. pectorale* have significantly higher swimming speeds and lower sedimentation speeds in diluted media owing to the decrease in cell size, but there is no difference in propulsion force between the treatments.

In contrast, *V. carteri* and *V. barberi* displayed intriguing phenotypic changes when grown for a short period in a low-nutrient medium. Regardless of treatment, *V. barberi* has a higher cell concentration per unit area and longer flagella than *V. carteri* (table 1). The initial experiments (table 1) showed that colonies grown in diluted media had smaller somatic cells with longer flagella and larger spheroids for both species, the latter owing to an increased amount of ECM. We investigated in more detail the phenotypic alterations of *V. carteri* (table 2), by using equipment that allowed measurements of the peak fluid speed $U$, the flagellar beating frequency $f$ and the force exerted by the flagella on the fluid $F$. Because in these more detailed experiments colonies were grown with lower light intensity (approx. 600 fc instead of approx. 1000 fc), they had fewer cells, and reached a smaller spheroid, cell and flagellar size than in the initial experiments. However, the data from the more detailed experiments had a better statistical significance and qualitatively confirm the results from the initial experiments (see comparison in figure 4). It is worth noting that these experiments showed that, regardless of nutrient treatment, colonies with longer cell concentration have longer flagella, and that colonies with longer flagella have a lower beating frequency (table 4). Further, results from these more detailed experiments showed that there was no difference in fluid speed $U$ between treatments, even though the biflagellated somatic cells were more sparsely spaced in the diluted medium owing to the larger spheroid size under those conditions. As there is only very weak evidence for a small increase in flagellar beating frequency upon dilution (table 2), the fact that the flow speed $U$ remains constant despite
with a larger number of flagellated cells that colonies with more cells had smaller cells with longer flagella regardless of treatment. In C. reinhardtii, C. reinhardtii takes the form colonies with more cells, somatic cells were smaller. Because V. carteri had smaller colonies with more cells, we found that colonies with more cells had smaller flagella regardless of treatment. In Volvox, we found that colonies with a larger number of flagellated cells Nc had a larger radius R and a larger cell concentration per unit area C. In V. barberi colonies with more cells, somatic cells were smaller. Because V. barberi germ cells perform binary fission, it was not possible to measure the germ cell size accurately.

Table 3. Model results for the MLR analysis of experiments conducted with populations grown at a light intensity of 1000 fc, i.e. experiment described in §2.1. The notation is as in table 1. Additional symbols are the total number of flagellated cells in colonies Nc, the change ∆ of the measured quantity over 8 h in normal medium and ∆ + δ in diluted medium. Data analysis takes the form d = d0 + αi + ∑ j aij + bjNc, with dummy indicator variables αi = 0, 1 to take account of nominal factors such as the medium treatment, and a, b and d0 are parameters of the model. A § marks statistically non-significant terms. Differences between replicated experiments were taken into account as a nominal factor and are not reported. In G. pectorale, we found that colonies with more cells had smaller colonies with longer flagella regardless of treatment. In Volvox, we found that colonies with a larger number of flagellated cells Nc had a larger radius R and a larger cell concentration per unit area C. In V. barberi colonies with more cells, somatic cells were smaller. Because V. barberi germ cells perform binary fission, it was not possible to measure the germ cell size accurately.

Table 4. Model results for the MLR analysis (as explained in table 3) of experiments described in §§2.2 and 2.3 conducted with populations of V. carteri, C. reinhardtii and G. pectorale grown at a light intensity of 600 fc. The notation is as in table 3. For V. carteri, the continuous variable T ranges from 1 to 4 to account for the four intervals of 30 min where data were recorded. A § marks statistically non-significant terms. As in the previous analysis, an increase in Nc increases R and C. Moreover, we found that colonies with more cells had smaller rs, larger U and larger F. In the 2 h measurement window, the colony spheroid, germ cells and flagella continued growing and the cell concentration continued decreasing significantly with time. Interestingly, the flagellar length l was significantly smaller when the cell concentration was large, regardless of treatment. The flagellar beating frequency f was found to be lower when the flagellar length was larger.

the increased cell separation implies that the increase in flagellar length provides the necessarily increased beating force F. Also, weak evidence from the detailed experiments on V. carteri suggests that the upward swimming speed (estimated V_up) increased for colonies grown in the diluted medium (table 2 and figure 4). In

<table>
<thead>
<tr>
<th>C. reinhardtii</th>
<th>d0</th>
<th>Δ</th>
<th>δ</th>
<th>Nc</th>
<th>r²</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>r_c (µm)</td>
<td>3.43 ± 0.12</td>
<td>1.46 ± 0.18</td>
<td>-0.36 ± 0.18</td>
<td>---</td>
<td>0.58</td>
<td>29</td>
</tr>
<tr>
<td>l (µm)</td>
<td>10.18 ± 0.28</td>
<td>-1.50 ± 0.39</td>
<td>§</td>
<td>---</td>
<td>0.43</td>
<td>16</td>
</tr>
<tr>
<td>G. pectorale</td>
<td>r_c (µm)</td>
<td>5.58 ± 0.44</td>
<td>0.82 ± 0.25</td>
<td>-0.34 ± 0.16</td>
<td>-0.14 ± 0.06</td>
<td>0.45</td>
</tr>
<tr>
<td>l (µm)</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>0.57 ± 0.25</td>
<td>0.17</td>
<td>5</td>
</tr>
<tr>
<td>V. carteri</td>
<td>R (µm)</td>
<td>126 ± 8.1</td>
<td>46 ± 5.4</td>
<td>14.6 ± 4.86</td>
<td>0.013 ± 0.003</td>
<td>0.65</td>
</tr>
<tr>
<td>r_c (µm)</td>
<td>23.2 ± 0.53</td>
<td>6.1 ± 0.70</td>
<td>-1.16 ± 0.63</td>
<td>§</td>
<td>0.53</td>
<td>29</td>
</tr>
<tr>
<td>r_s (µm)</td>
<td>3.81 ± 0.08</td>
<td>0.79 ± 0.10</td>
<td>-0.46 ± 0.09</td>
<td>§</td>
<td>0.58</td>
<td>34</td>
</tr>
<tr>
<td>l (µm)</td>
<td>14.28 ± 0.58</td>
<td>3.03 ± 0.77</td>
<td>2.14 ± 0.68</td>
<td>§</td>
<td>0.48</td>
<td>23</td>
</tr>
<tr>
<td>C (cells/10^3)</td>
<td>3.07 ± 0.56</td>
<td>-2.88 ± 0.37</td>
<td>-0.65 ± 0.33</td>
<td>0.021 ± 0.0002</td>
<td>0.69</td>
<td>48</td>
</tr>
<tr>
<td>V. barberi</td>
<td>R (µm)</td>
<td>170 ± 15.0</td>
<td>60 ± 10.5</td>
<td>59.1 ± 11.0</td>
<td>0.009 ± 0.002</td>
<td>0.82</td>
</tr>
<tr>
<td>r_s (µm)</td>
<td>6.11 ± 0.28</td>
<td>1.88 ± 0.20</td>
<td>-0.39 ± 0.21</td>
<td>-0.0003 ± 0.0001</td>
<td>0.82</td>
<td>109</td>
</tr>
<tr>
<td>l (µm)</td>
<td>24.80 ± 3.51</td>
<td>5.80 ± 2.46</td>
<td>9.93 ± 2.56</td>
<td>§</td>
<td>0.63</td>
<td>22</td>
</tr>
<tr>
<td>C (cells/10^3)</td>
<td>4.99 ± 0.93</td>
<td>-3.85 ± 0.65</td>
<td>-1.20 ± 0.57</td>
<td>0.0008 ± 0.0002</td>
<td>0.81</td>
<td>65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. reinhardtii</th>
<th>d0</th>
<th>δ</th>
<th>Nc (10^-3)</th>
<th>T</th>
<th>C</th>
<th>l</th>
<th>r²</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>r_c (µm)</td>
<td>5.90 ± 0.11</td>
<td>-1.25 ± 0.16</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.42</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>V_med (µm s^-1)</td>
<td>7.18 ± 0.35</td>
<td>-2.75 ± 0.27</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.32</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>V_up (µm s^-1)</td>
<td>42.8 ± 2.45</td>
<td>11.4 ± 3.32</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.05</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>G. pectorale</td>
<td>r_c (µm)</td>
<td>6.61 ± 0.18</td>
<td>-0.31 ± 0.14</td>
<td>-220 ± 30</td>
<td>---</td>
<td>---</td>
<td>0.31</td>
<td>11</td>
</tr>
<tr>
<td>V_med (µm s^-1)</td>
<td>11.0 ± 0.39</td>
<td>-1.11 ± 0.54</td>
<td>§</td>
<td>---</td>
<td>---</td>
<td>0.02</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>V_up (µm s^-1)</td>
<td>32.5 ± 1.35</td>
<td>4.21 ± 1.76</td>
<td>§</td>
<td>---</td>
<td>---</td>
<td>0.02</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>V. carteri</td>
<td>R (µm)</td>
<td>112 ± 7.6</td>
<td>27.2 ± 3.32</td>
<td>16 ± 4</td>
<td>5.29 ± 1.49</td>
<td>---</td>
<td>0.75</td>
<td>37</td>
</tr>
<tr>
<td>r_c (µm)</td>
<td>26.0 ± 0.70</td>
<td>§</td>
<td>---</td>
<td>0.40 ± 0.26</td>
<td>---</td>
<td>0.35</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>r_s (µm)</td>
<td>5.13 ± 0.14</td>
<td>-0.25 ± 0.07</td>
<td>-0.27 ± 0.09</td>
<td>§</td>
<td>---</td>
<td>0.41</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>l (µm)</td>
<td>19.18 ± 0.59</td>
<td>0.93 ± 0.25</td>
<td>§</td>
<td>0.44 ± 0.10</td>
<td>-0.32 ± 0.05</td>
<td>---</td>
<td>0.67</td>
<td>25</td>
</tr>
<tr>
<td>C (cells)</td>
<td>4.23 ± 0.77</td>
<td>-2.57 ± 0.34</td>
<td>4 ± 0.4</td>
<td>-0.48 ± 0.15</td>
<td>---</td>
<td>0.70</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>per 10^3 µm^2</td>
<td>f (Hz)</td>
<td>39.3 ± 2.45</td>
<td>0.66 ± 0.45</td>
<td>§</td>
<td>§</td>
<td>---</td>
<td>-0.70 ± 0.14</td>
<td>0.26</td>
</tr>
<tr>
<td>U (µm s^-1)</td>
<td>368 ± 21</td>
<td>68 ± 11</td>
<td>-10.1 ± 4.23</td>
<td>§</td>
<td>---</td>
<td>0.58</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>F (pN)</td>
<td>1249 ± 105</td>
<td>211 ± 51</td>
<td>443 ± 64</td>
<td>§</td>
<td>---</td>
<td>0.68</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>V_up (µm s^-1)</td>
<td>274 ± 10.2</td>
<td>26 ± 14.4</td>
<td>§</td>
<td>---</td>
<td>---</td>
<td>0.46</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>
the diluted medium, colonies have smaller somatic cells
(i.e. lower negative gravitational force) and longer
flagella (i.e. larger swimming force). These benefits
seem to outweigh the increase in drag owing to the
larger spheroid of colonies grown in diluted medium.

A plausible interpretation of the results showing that
Volvox colonies, when grown in a diluted medium, make
investments into collective properties, such as a larger
spheroid radius R and maintaining a high fluid speed
U, is that these changes tend to increase the rate of
nutrient uptake and thereby help compensate for the
environmental change. There are two key physical
aspects that must be considered in estimating the rate
of nutrient uptake to a spherical organism like Volvox.
The first is the fact that the absorbing somatic cells
cover only a fraction of the total colony surface, and it
is not obvious a priori how even the purely diffusive
rate of uptake would depend on the somatic cell size
and the overall colony radius in a geometry with
such patchy absorbers. However, this is precisely the
problem considered some time ago in the context of
chemoreception [28]. There it was found that the
absorption rate J to a sphere of radius R whose surface
is covered by a absorbing discs, each of radius rS, is
J \propto n_rS/(n_rS + \pi R), where J_{\text{max}} \approx 4\pi D_{\text{max}} R is the rate
associated with a sphere whose entire surface is a per-
fected absorber. When the number of discs tends to
infinity the rate sensibly approaches J_{\text{max}}, but the key
point is that it can be very close to this asymptotic
value even for moderate coverage of the surface. (The
first detailed discussion of this kind of effect was given
by Jeffreys [29] in the context of evaporation from the
stomata on leaves.) Expressing the result as
J = \int_0^R \int_{\text{D}_{\text{wall}}} dC_{D_{\text{max}}} R/(1+\pi R/n_{rS}), and using the values typical of
Volvox (n = 1000, R = 250 \mu m, rS = 5 \mu m), the
ratio \pi R/n_{rS} \sim 0.16 and thus J/J_{\text{max}} \approx 0.86, only
slightly depressed from the asymptotic value. We
conclude from this that the surface coverage of somatic
cells in Volvox is sufficiently large that not only is the
diffusive rate of absorption well approximated by that of
a sphere absorbing over its entire surface, but even
quite substantial increases in the colony radius still
leave it in that regime, so the purely diffusive absorp-
tion rate actually increases with colony radius at fixed
somatic cell number. This would not be the case for
very small N_{rS} (\pi R/N_{rS} \gg 1), for then the rate is
simply proportional to N_{rS} and independent of the
colony radius R. Using the typical values of R and rS
above, this would require n \ll 150.

The second issue to consider is how the presence of a
fluid flow past the colony surface might affect the
results described above. While there has been no
detailed mathematical analysis of this particular prob-
lem, we may draw some conclusions based on the
typical flow rates and diffusivities. The key physical
feature that results in the diffusive flux in the abscence
of flow being so close to the fully absorbing sphere value
is the very large number of encounters that a diffusing
molecule makes with the sphere when it is in the vicinity
of the surface [28]. It follows then that advection
parallel to the surface would not significantly alter
this effect (in fact it may even enhance it) provided
the time spent near the surface during advection is
not severely curtailed. In the case of Volvox the time
scale for advection along the colony surface is several
seconds, and in that time a molecule would typically
diffuse a distance (2D_{\text{max}})^{1/2} \sim 40 \mu m, a distance large
compared with the somatic cell size and comparable
to if not greater than the intersomatic cell spacing.
Thus, as the molecules are swept over the surface,
they indeed have sufficient time to find an absorbing
somatic cell.

The arguments advanced above suggest that nutrient
uptake for an organism like Volvox can be estimated on
the basis of a fully absorbing sphere. Attention then
turns to the rate of uptake at high \text{Pe}clet numbers. For a Volvox spheroid, \text{Pe} = 2R U/D,
as the typical length scale over which the self-generated
flow changes is 2R [5]. Increasing R and maintaining a
high U may thus be seen as a strategy for Volvox to
maintain, or even increase, the high \text{Pe}. Such a strategy
is beneficial for the colony, as the rate of nutrient
uptake by a ciliated spherical micro-organism through
its surface is predicted to be proportional to \text{R}^3/2
[5,30]. Qualitatively, this \text{Pe} dependence of the nutrient
uptake rate can be understood by noting that the high
flow speeds create a fluid-dynamical boundary layer
above the spheroid surface across which there is a
steep nutrient concentration gradient, which leads to
an enhanced diffusive transport across the boundary
layer onto the organism surface. This strong depen-
dence on \text{Pe}clet number for large organisms should be
contrasted with that for small organisms. A variety of
calculations [5,30] suggest that, for organisms with a
small \text{Pe}clet number, the correction to the diffusive
uptake owing to fluid flow is linear in \text{Pe}. Thus, an
organism in the regime \text{Pe} \ll 1 will in general make
only a small change to its uptake rate by a fractional
change in \text{Pe}, whereas a comparable change in \text{Pe}
for \text{Pe} \gg 1 can produce a much larger change in uptake,
proportional to \text{Pe}^{1/2}. Even though Volvox is a colonial
organism without a central nervous system, the phenotypic
plasticity it displays suggests ‘awareness’ of the benefits
associated with collective behaviour. The self-generated
fluid flows are thus not only important for self-propulsion
and phototaxis [51], but also for nutrient uptake.

The efforts of Volvox to counteract an impending
decrease in nutrient uptake, if grown in low-nutrient
medium, have a positive effect on the growth of the
germ cells (which later turn into the daughter colonies;
figure 3) in V. carteri. In the initial experiments, there
was statistically weak evidence for a small dependence
of the germ cell radii rS on the nutrient treatment
(table 1), but there was no statistically significant
dependence of rS on the nutrient treatment in the
more detailed experiments (table 2). These results
suggest that, in the investigated time window of the
Volvox life cycle, colonies can maintain (almost) equal
germ cell growth rates in normal and 10^{-3} diluted media.

4. CONCLUSION

We found evidence that growth in low-nutrient medium
induces phenotypic plasticity that mitigates the effect
of nutrient limitation in large Volvocales (V. carteri

J. R. Soc. Interface (2011)
and V. barberi, and a lack of such plasticity in small Volvocales (C. reinhardtii and G. pectorale). The changes in phenotype induced by growing Volvox in a diluted medium were investments into advective fluid flows, and into an increase in colony radius. Such investments point to the important role of advection in enhancing nutrient uptake for the germ cells that grow inside the Volvox colony, consistent with recent theory [5] and experiments [16] which suggested a link between the Péclet number and the evolution to larger organism sizes and germ–soma differentiation in the Volvocales. Although this work provides further evidence for the importance of advection in nutrient uptake for large multicellular micro-organisms, direct measurement of the advection dependence of the rate of nutrient uptake or metabolic activity still require further study. Likewise, further studies are needed to understand the control of collective flagellar beating and the connection between flagellar beating frequency, length and spacing.

We are grateful to Matt Herron for a critical reading of the manuscript and many detailed suggestions, and thank J.-W. van de Meent, T. J. Pedley and I. Tuval for discussions. This work was supported in part by NSF grants DEB-0075296 (C.A.S., R.E.M.) and PHY-0551742 (S.G., J.O.K., R.E.G.), the Engineering and Biological Systems programme of the BBSRC and the Schlumberger Chair Fund.

REFERENCES