Motility, Mixing, and Multicellularity

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Abstract. A fundamental issue in evolutionary biology is the transition from unicellular to multicellular organisms, and the cellular differentiation that accompanies the increase in group size. Here we consider recent results on two types of "multicellular" systems, one produced by many unicellular organisms acting collectively, and another that is permanently multicellular. The former system is represented by groups of the bacterium *Bacillus subtilis* and the latter is represented by members of the colonial volvocalean green algae. In these flagellated organisms, the biology of chemotaxis, metabolism and cell-cell signaling is intimately connected to the physics of buoyancy, motility, diffusion, and mixing. Our results include the discovery in bacterial suspensions of intermittent episodes of disorder and collective coherence characterized by transient, recurring vortex streets and high-speed jets of cooperative swimming. These flow structures markedly enhance transport of passive tracers, and therefore likely have significant implications for intercellular communication. Experiments on the Volvocales reveal that the sterile flagellated somatic cells arrayed on the surface of *Volvox* colonies are not only important for allowing motion toward light (phototaxis), but also play a crucial role in driving fluid flows that transport dissolved molecular species. These flows, generated by the collective beating of flagella, confer a synergistic advantage with regard to transport of nutrients and chemical messengers. They allow these species to circumvent a nutrient acquisition bottleneck which would exist if transport were purely diffusive, and thereby evolve to larger multicellular individuals. In both cases, a higher level of organization, specialization and complexity counteract the higher costs inherent to larger groups.

Keywords: motility, mixing, multicellularity, chemotaxis

1 Introduction

The design of an "organism" intended to be efficient for a specific task, in a specific environment, must take into account interactions with that environment. Sometimes the environment is passive and homogeneous, such as still water in a puddle. It can be dynamic, driven by winds or currents, and inhomogeneous in its chemical contents or illumination. In the realistic biological context, it may contain hordes of similar or identical autonomous organisms, interacting with one another through the dynamics of their habitat, summarized in the present context by the Navier-Stokes and advection-diffusion equations [1]. Since the organism(s) must acquire and discharge food and waste molecules, under this variety of conditions, it must be designed to cope with all of them, whilst outcompeting rivals and escaping predators. This herculean task is accomplished by the process of evolution that generates ever greater competents and eliminates failures.

This paper is concerned with the fundamental issue of multicellular organisms interacting with their environments. Multicellularity can be achieved in two ways [2]: by a loose aggregation of unrelated cells such as in myxobacteria [3], or by the aggregation of mitotic clonal products that are held together by a cohesive extra-cellular material, as in eukaryotes. In the former case entities become closely spaced and behave as multicellular organisms [4, 5]; the latter case was the path taken by plants and animals. That natural selection has favored multicellularity is illustrated by its multiple independent origins (especially clear for the case of algae [6, 7]).

The fitness of the emerging multicellular unit can be analyzed in terms of its two basic components: fecundity (rate of reproduction) and viability (survival) [8, 9]. In both the emergence of a complex multicellular individual in eukaryotes, and the emergence of a group of cells by aggregation in prokaryotes, benefits and costs arise to fecundity and viability as the multicellular individual or group increases in size. Furthermore, in general an organism that invests more resources in fecundity-related functions detracts from viability-related functions and vice versa, the trade-off dynamic changing as a function of group size [9]. The formation of a large multicellular individual or group can be beneficial both for viability (in terms of predation avoidance, ability to catch larger prey, creation of a buffered environment within a group) and fecundity [10, 11]. But large size can also become costly, both in terms of viability, as having an increased need for local resources and increased accumulation of waste products, and fecundity, through increased generation time. As size increases, such costs negatively affect the fitness of the multicellular individual or the cell group. Thus, we argue that the key factor in generating higher-level organization (multicellularity) and complexity (cell specialization) from lower level units (cells) may be the conflicts/tensions/struggles and trade-offs between reproducing (fecundity), surviving (viability), and acquiring resources as a group increases in size.

As group size increases, how do organisms in the uni-multicellular transition deal with the increase in transport costs? How does increasing their investment in locomotion and mixing affect viability? How do they alter their mode of reproduction? In this paper we present examples in which the transition to multicellularity as well as the aggregation of prokaryotes happened/happens in aqueous environments not subject to exogenous stirring. To survive and reproduce in such still environments, where resources are not continuously mixed, these larger multicellular organisms or bacterial cell groups have to deal with two types of transport: (i) translocation of themselves to reach essential resources, and (ii) transport, whether passive or active, of the resources from the surrounding environment to the inside of the organism, and transport waste from the inside to the outside. Many species of bacteria and algae use the propulsion generated by the rotation or beating of their flagella to reach resources and better environments.

In some cases, these flagellar motions are also associated with improving the acquisition of essential molecules important for survival, and with dispersal of waste products and communication signals beyond the range of inadvertent diffusive recycling. Here we use the bacterium *Bacillus subtilis* [12] and the volvocalean green algae [13] as illustrative examples. We suggest that these results have important implications for computational models of the evolution of multicellularity [14, 15, 16, 17] and such specific behaviors as "flocking" [18]. They also provide a context within which to link developmental issues, such as reproductive modes, with evolutionary features.

2 Low Reynolds Environments and Transport Limitations

If a molecular species dissolved in a fluid (e.g., oxygen) diffuses in space and is carried by fluid motion (the process of advection), and the fluid has density ρ, pressure p, and viscosity η, then the fluid velocity $\mathbf{u}$ and concentration C evolve in time as [1]

$$\mathbf{u}_t + \mathbf{u}\cdot\boldsymbol{\nabla}\mathbf{u} = -\frac{1}{\rho}\boldsymbol{\nabla}p + \nu\nabla^2\mathbf{u} \ , \tag{1}$$

and

$$C_t + \mathbf{u}\cdot\boldsymbol{\nabla}C = D\nabla^2 C \ , \tag{2}$$

where $\nu = \eta/\rho \sim 0.01$ cm^2/s is the kinematic viscosity of water. and D is the diffusion coefficient (typically $\sim 10^{-5}$ cm^2/s for small molecules such as oxygen and considerably smaller for large ones, e.g. polysaccharides). If U is a characteristic fluid velocity, varying on a length scale L, then the ratio of the inertial and viscous terms defines the Reynolds number Re,

$$Re = \frac{\mathbf{u}\cdot\boldsymbol{\nabla}\mathbf{u}}{\nu\nabla^2\mathbf{u}} \sim \frac{U^2/L}{\nu U/L^2} \sim \frac{UL}{\nu} \ . \tag{3}$$

This dimensionless quantity can be viewed as a ratio of the time for diffusion to that for advection. The advection time for a parcel of fluid to be carried a distance L by a flow U is L/U, and time for a velocity to spread out by viscosity is L^2/ν. Likewise, the ratio of advection to diffusion defines the Peclet number Pe [1],

$$Pe = \frac{\mathbf{u}\cdot\boldsymbol{\nabla}C}{D\nabla^2 C} \sim \frac{UC/L}{DC/L^2} \sim \frac{UL}{D} \ , \tag{4}$$

where now the comparison is between an advection time L/U and the diffusion time L^2/D. If the length scale is that of an individual bacterium, $L \lesssim 10^{-3}$ cm, and the fluid velocity is a characteristic swimming speed, $U \sim 10^{-3}$ cm/s, we see that $Re \lesssim 10^{-4}$; individual microorganisms live in a world of vanishingly small Reynolds numbers, the "creeping flow" or "Stokes" regime, where the left-hand-side of Eq.1 is negligible and motion is dominated by friction. This is very much unlike what we experience in a swimming pool. There is no coasting, so when appendages stop moving, so does the organism. Note also that in the absence of the left-hand-side of Eq. the equation is linear and without any explicit time derivative. This, in the presence of rigid boundary conditions, implies a type of reversibility which constrains the types of swimming strokes which produce net motion [19]. Only those which themselves break time reversal invariance will work, such as a traveling wave on an elastic filament [20].

By similar reasoning, $Pe \sim 10^{-2}$ for small solutes, and transport is dominated by diffusion. When $Pe < 1$, diffusion outcompetes transport by advection from the flowing medium, whereas if $Pe > 1$, advection dominates. While linearity and reversibility are relaxed when an organism can periodically change its shape or includes rotating helices, the dominant role of diffusion is the (correct) basis of conventional wisdom regarding the dynamics of single organisms on the micron scale [21].

The situation changes radically when many closely spaced, moving entities - motile bacteria, or flagella on the surface of an algal colony - collectively generate flows. We have recently demonstrated [22, 23, 24, 25] that such flows have great speeds, exceeding the typical swimming speeds of individual organisms, and can persist over very large distances, implying that $Pe \gg 1$. They may also exhibit long correlation lengths and times, and can be chaotic, exhibiting stretching and folding that enhances local mixing. Thus, organisms that can concentrate themselves, such as *Bacillus subtilis*, and organisms covered by arrays of flagella, such as the volvocalean green algae, are freed from the tyranny of transport by diffusion only. The apparatus conferring motility can then also improve molecular transport of nutrients, waste products and chemical messengers.

3 The Transition to Multicellularity in Volvocales

Volvocalean green algae are an ideal model system for studying the transition from unicells to multicellular organism with cells specializing in vegetative (soma) and reproductive (germ) functions since they comprise an assemblage of lineages featuring varying degrees of complexity in terms of colony size, colony structure, and cell specialization. They range (Fig. 1) from the unicellular biflagellated *Chlamydomonas* to colonies made of 4-64 cells with no cellular differentiation, e.g., *Gonium* and *Eudorina*, to multicellular individuals comprising 1,000-50,000 cells with complete specialization in reproductive and vegetative functions (germ-soma separation, as in *Volvox* [13, 26, 27, 28]). In the multicellular forms, each of the *Chlamydomonas*-like somatic cells is found at the surface of an extracellular matrix, with its two flagella oriented outwards. Germ-soma separation characterizes the large members of this lineage. The number of somatic cells (N_S) per reproductive cell (N_R; the N_S/N_R ratio) increases with colony size [27].

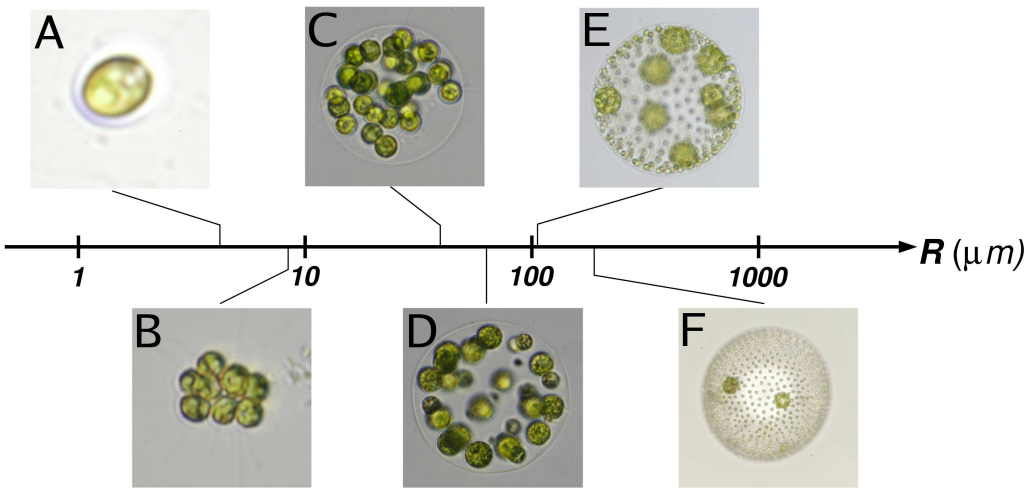

Figure 1. Volvocalean green algae arranged according to typical colony radius R. The lineage ranges from the single-cell *Chlamydomonas reinhardtii* (A), to undifferentiated *Gonium pectorale* (B), *Eudorina elegans* (C), to the soma-differentiated *Pleodorina californica* (D), to the germ-soma differentiated *Volvox carteri* (E), *V. aureus* (F), and even larger (e.g. *V. gigas* with a radius of 1 mm). In species in which two cell types can be identified, the smaller are somatic cells and the larger are reproductive cells. Note that the number of cells in *Volvox* species ranges from $1,000$ (e.g. *V. carteri*) to $50,000$ (e.g. *V. barberi*).

Although the precise relationships among species are not well resolved in the Volvocales, one inference repeatedly emerges: *Volvox* species (Fig. 1) with increased cell specialization do not have a single origin (i.e., monophyletic). These complex forms have evolved several times independently, from quite different ancestors [29, 30, 31, 32]. Lineages exhibiting the different developmental programs [33] (differing in details of cell division) are interspersed with each other and with non-*Volvox* species, indicating that they have also evolved several times independently. Supporting this evidence for ease of evolutionary transition in the Volvocales is the underlying genetic architecture responsible for the separation of germ and soma, which does not involve many genetic steps [34]. Only two mutations are required to transform *V. carteri* into a mutant (*V. carteri* $glsA^{-}/regA^{-}$) with morphological and life-history features similar to those of *Eudorina* colonies with no cellular differentiation [35] Likewise, a mutant of *V. powersii* morphologically identical to a member of the genus *Pleodorina* has also been described [36]. In short, the Volvocales comprise a group of closely related lineages with different degrees of cell specialization which seem to represent "alternative stable states" [37].

Volvocalean green algae are found in quiet, standing waters of transient vernal puddles or in permanent lakes when thermal stirring stops and the lake becomes stratified [13, 38]. Since they are negatively buoyant, these organisms need flagellar beating to avoid sinking and to reach light and nutrients [13, 27]. Change in the flagellar apparatus between unicellular species and species that form colonies is still further evidence of how important motility is for the Volvocales [39].

Thus, the constraints and opportunities of flagellar motility may have been the major driving force as colonies increased in size during the evolutionary transitions from multicellular colonies with no cellular differentiation to multicellular colonies with germ-soma separation.

Below we present a feature that is critical to the evolution of multicellularity in this group. In the Volvocales, cells do not double in size and then undergo binary fission as in almost all the other lineages. Rather, each cell grows about $2n$-fold in size, and then undergoes a rapid, synchronous series of n divisions (under the mother cell wall). This type of cell division is known as palintomy with multiple fission. Palintomy occurs in all the smaller Volvocales (from unicellular *C. reinhardtii* to *Pleodorina* colonies with only soma specialization ($64 - 128$ cells; Figure 1), and in all the members of the Merillosphaera *Volvox* group (e.g., *V. carteri*; colonies with complete germ-soma differentiation; $500 - 4000$ cells; Figure 1). Palintomy with multiple fission is considered an ancestral feature in this group and has likely predisposed these algae to multicellularity. In *Chlamydomonas*, the cells ($2^2 - 2^4$ cells) separate from each other after division. However, in the other volvocalean species, the cluster of 2^n cells does not disintegrate, and colonial forms are produced [13]. This manner of colony formation implies that the cells in the adult colony are clonally derived from a single cell after a specific number of cell divisions, n ($n = 3$ for *Gonium*; $n = 5$ for *Eudorina*, $n = 6 - 7$ for *Pleodorina* and $n = 8 - 12$ for *Volvox*). Therefore, in the Volvocales, conflict derived from within-group variation is likely to be of no consequence because of the "parental control" on the cell phenotype. In short, the cell fate is determined during development, under the control of the "mother" cell.

V. carteri is the species that best illustrates the genetic mechanisms of cell differentiation in the Volvocales [13, 40]. It is thought that in *V. carteri* the mechanism of cellular differentiation involves only three genes. The *V. carteri* palintomic germ cell (gonidium) first grows to a size that allows it to perform all the cell divisions necessary to produce a daughter colony. Once it reaches that size, it first performs five symmetric divisions, producing 32 cells of the same size. After that the *Gls* gene is turned on and the gonidium performs a series of asymmetric divisions, producing a small number of larger cells that will become the germ line, and a large number of small cells that will become the soma. Interestingly, a size threshold determines the fate of each cell. In the small cells the *RegA* gene is turned on, which suppresses the reproductive functions. These cells are permanently flagellated, only performing motility functions through the lifespan of the colony until they undergo programmed cell death. In contrast, in the large cells the *Lag* gene is turned on, which suppresses motility functions. These cells become the germ line and lack any flagella and eyespot for phototactic orientation.

To summarize, in single cell organisms (i.e. *Chlamydomonas*), one cell must perform both basic functions for fitness: survive (e.g., motility) and reproduce, typically these two functions being separated in time. In *V. carteri*, via parental manipulation by the gonidium of gene expression, some cells specialize into one component (e.g., reproduction) by losing the other (e.g., motility) and vice versa, leading naturally to an "unsophisticated" division of labor and leading to the differentiation of germ and soma.

In Volvocales, because of their coherent glycoprotein rigid cell wall, the basal bodies cannot move laterally and take the position expected for centrioles during cell division while still remaining attached to the flagella (as they do in naked green flagellates). Consequently, in undifferentiated colonies, motility is inhibited during cell division. This inability to both divide and maintain flagellar activity is referred to as the "flagellation constraint" [27]. As the number of cells in the colonies increases, the time spent in the division phase increases since each reproductive cell has to produce larger colonies. Therefore, since Volvocales are negatively buoyant,

the motility function so basic to survival is increasingly compromised. Because a flagellum may beat for up to 5 cell divisions without the basal bodies attached, the 32 cell colony size (*Eudorina*) seems to be the critical threshold at which motility is severely compromised. Koufopanou [27] argued that the evolution of permanently flagellated sterile cells (soma) in colonies larger than 32 cells, and hence the evolution of germ-soma separation, was because of the "flagellation constraint."

To further investigate if the hydrodynamic opportunities and costs of increased size may have been the major driving force during the evolutionary transitions from colonies with no cellular differentiation to true multicellular individuals with germ-soma separation, a hydrodynamic model was developed and the swimming capabilities of various volvocalean species and mutant forms were studied [28]. In this context, such a model is a mathematical formulation of the relationship between the forces induced by the beating of flagella, the fluid flows they create, and the swimming speed that results through a balance against drag and buoyancy. Since volvocalean algal colonies are small-diameter spheroids that swim at low velocities, they can be modeled as moving spheres in the low Reynolds number regime described above. Even for the largest *Volvox* colonies, the Reynolds number is considerably less than unity. In this regime, the drag force F on a moving self-propelled sphere can be approximated by the well-known Stokes results $F = 6\pi\eta Rv$ for a solid sphere of radius R. Within this same framework, the force exerted by a colony swimming vertically upward at a specific velocity (v_{up}) balances the sum of the drag force and that of gravity, $Nqf = 6\pi\eta Rv_{\mathrm{up}} + g\Delta m$, where N is the number of cells, q the proportion of flagellated cells arrayed on the surface of the colony, f the average upward swimming force per flagellated cell, Δm is the difference in mass between the colony and the displaced water, and g is the acceleration of gravity.

This hydrodynamic model shows that larger colonies need to invest in somatic cells that specialize in motility in order to remain motile and avoid sinking while reproducing (Figure 2) [28]. As colonies increase in size, a higher proportion of somatic cells is required (i.e., an increased somatic to reproductive cell ratio N_S/N_R). This need arises from the enlargement of reproductive cells to form daughter colonies that increase the mean mass of the colony. Since the number of cells is not augmented during adulthood, the colonies have a fixed number of motile cells. Therefore, there is no added propulsion for moving a colony that becomes larger as it develops. Once larger colonies have a high proportion of somatic cells, they are better off with a non-flagellated germ cell that specializes in reproduction. As N_S/N_R increases, the benefit to viability of having motile reproductive cells declines due to the decrease of the proportion of reproductive cells in the colony. A specialized non-flagellated germ cell will presumably have available more resources for reproduction. Therefore, increased germ-soma specialization allows larger colonies to reach an even higher fitness level by enhancing both motility and colony productivity.

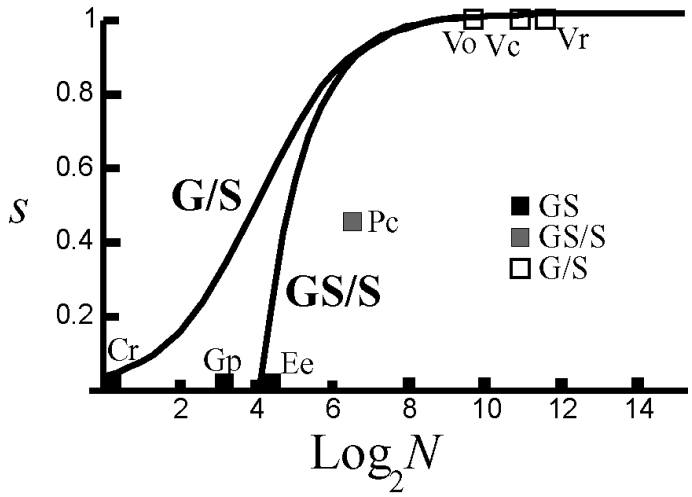

Figure 2. Results of the hydrodynamic model: Proportion s of somatic cells needed by colonies of *C. reinhardtii* -type cells to avoid sinking. Model results for colonies with unspecialized reproductive cells (GS) and soma (S; GS/S colonies) and for colonies with specialized germ (G) and soma (G/S colonies) are plotted as a function of number of cell divisions ($\mathrm{Log_2N}$). When $s = 0$, colonies only have undifferentiated GS cells. For colonies to avoid sinking as they increase in size, they must invest in a higher proportion s of somatic cells. Note that for smaller colonies, GS/S colonies need a smaller proportion of somatic cells than G/S colonies to stay afloat since GS cells are flagellated. As size increases, the difference between the two colony types becomes negligible because the proportion $(1 - s)$ of reproductive cells becomes very small. Some of the extant species data is plotted to show how it fits with the model. Cr: *C. reinhardtii*; Gp: *G. pectorale*; Ee: *E. elegans*; Pc: *P. californica*; Vc: *V. carteri*; Vo: *V. obversus*; Vr: *V. rousseletii*. GS: undifferentiated colonies; GS/S: soma differentiated colonies; G/S: germ-soma differentiated colonies. Although *V. rousseletii* colonies reproductive cells start as flagellated cells, their flagella have essentially no motility function since they are reabsorbed before the first cell division. Thus, we consider *V. rousseletii* reproductive cells non-flagellated (G) and *V. rousseletii* G/S colonies. Figure adapted from Solari, et. al. [28].

Moreover, it was shown both experimentally [24] and theoretically [25] that investment in somatic cells for motility has the additional and important benefit of enhanced molecular transport of nutrients and wastes thanks to the flow created by collective flagellar beating. To make this point clear, Figure 3 shows two views of the flow fields generated by flagella around a *Volvox* colony held fixed. The left panel is a time exposure of several seconds showing the paths of tracer particles advected by the flow. Superimposed on a laminar background are seemingly chaotic vortical structures near the colony surface. The right-hand panel shows the streamlines in a wider field of view obtained by averaging over a considerably longer period of time. Chaotic details are averaged out, leaving a very large scale laminar flow extending many colony diameters. The velocities of these fluid flows can approach $1,000$ μm/s, on scales of $1,000$ μm, producing Peclet numbers in the several hundreds. Thus, for long range transport, advection strongly dominates diffusion and the quantitative rates of nutrient acquisition and waste removal are dramatically

different than those based on diffusion-limited uptake. Indeed, since the diffusion-limited current scales linearly with the colony radius, whereas the current of needed metabolites scales as the surface area (when the metabolic tissue is only on the surface, as in the non-differentiated colonies), there is a bottleneck radius beyond which the needs outstrip the supply. Theoretical calculations [25] show, quite remarkably, that the nature of a concentrative boundary layer formed near the colony surface at high Peclet numbers renders the advectively-enhanced currents quadratic in the colony radius, thus bypassing that bottleneck. In other words, there is an advantage to the individuals which derives from the *collective* fluid flows they produce. Furthermore, experiments show that when permanently deflagellated colonies were placed in still medium there was a significant decrease of the average germ cell diameter growth, but when permanently deflagellated colonies were placed in artificially mixed medium, they grew as well as normal flagellated colonies [24]. These results clearly confirm that flagella generate important transport and mixing flows that aid in resource uptake and waste removal. It should be emphasized that the rates of nutrient uptake and waste removal vary significantly during the life of these organisms, as photosynthetic rates change diurnally and the organisms age. As there are different biochemical pathways for the various molecular species that are taken up and given off, both inward and outward fluxes can coexist in time.

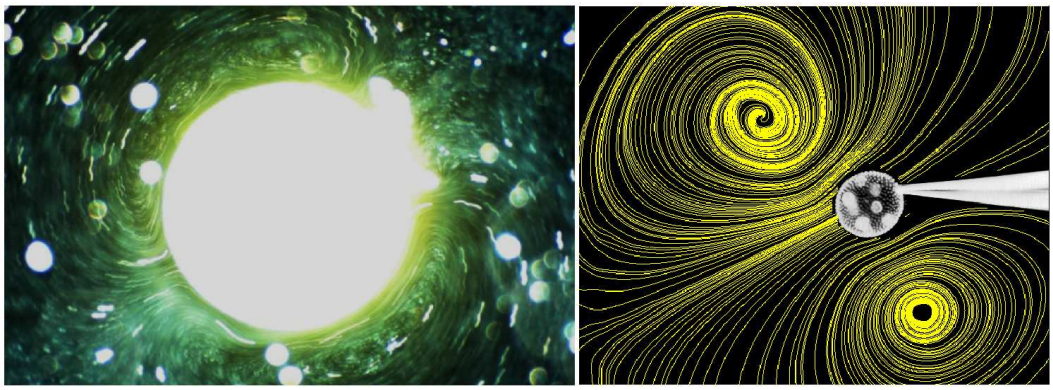

Figure 3. Flow fields around *Volvox carteri* colonies approximately 300 microns in diameter. Left panel is a time exposure of several seconds which reveals the complex flows of tracers near the colony surface. Right panel shows streamlines obtained from a time series of many tens of seconds around a colony held fixed with a glass micropipette.

In conclusion, as selective pressures first pushed volvocalean colonies to increase in size, the costs of reproducing an increasingly larger group also increased, having increasingly negative effects on the fitness of the colonies. Beyond definite threshold sizes, volvocalean colonies sink and their nutrient uptake is constrained, presumably dramatically decreasing viability. According to insights afforded by our models [25, 28], overcoming these thresholds requires the separation of reproductive (germ) and motility (soma) functions between two cell types, resulting in increased cell specialization as colony size increases, making larger colonies viable and creating a true multicellular individual.

4 Collective Dynamics in Bacterial Suspensions

As discussed in the previous section, the thousands of flagella affixed to the periphery of *Volvox* colonies rather remarkably fulfill two distinct functions, both necessary for the "success", the *viability*, of individual coherently functioning multicellular organisms. They serve to provide locomotion and transport, enhanced significantly beyond molecular diffusion, yielding sufficient arrival of required solutes and elimination of waste products.

Volvox is the apex of an evolutionary sequence that starts with single cells and continues on to higher multiples, forming colonial individuals. Are there more "primitive" circumstances where single cells act collectively [41], functioning as a sort of intermittently coherent individual, without actually being permanently attached to one another? We describe one striking example exhibited by the common soil bacteria *Bacillus subtilis* [12]. These rod-shaped prokaryotes range from $4-6$ μm in body length, depending on conditions and growth phase. Under optimum conditions, the doubling time is about 20 minutes. They are peritrichously flagellated, i.e their helical, $10-15\mu$m long flagella are attached to rotary motors distributed somewhat randomly over the cell body. The flagella are driven at ~ 100 rps, producing a cell swimming speed up to $\sim 30\mu$m/s. Individual cells are characterized by $Re \ll 1$ and $Pe < 1$. They are in the reversible, linear regime of Stokes flow; molecular transport toward and away from a cell is diffusive.

The situation changes when the chemical content of their habitat is favorable and when, given appropriate geometries, the concentration of these bacteria becomes very large, approximately close-packed, somewhat like wiggly nails in a bucket. *B. subtilis* is an obligate aerobe, and in a suspension will swim toward the fluid-air interface, up the oxygen concentration gradient that they produce by their respiration. Since their density is slightly greater than that of water, this surface accumulation leads to a striking instability known as bioconvection (Fig. 4A) [41], which in suitable geometries produces spontaneous "self-concentration" into nearly close-packed arrangements (such as near the contact line, Fig. 4B). There, the cells exhibit dynamic, correlated steric alignment much like a nematic liquid crystal. The flagella rapidly and coherently move these transient, recurring domains, each containing thousands of cells. We have named this phase the "Zooming Bio-Nematic" [42]. Studies of tracer particle dynamics to characterize fluid flow are straightforward (Fig. 4C). The ZBN phase is ideally suited for investigating rapid long range intercellular molecular communication, associated with quorum sensing [43] and biofilm formation [44, 45]. This phase shares some properties with swarming populations [46, 47].

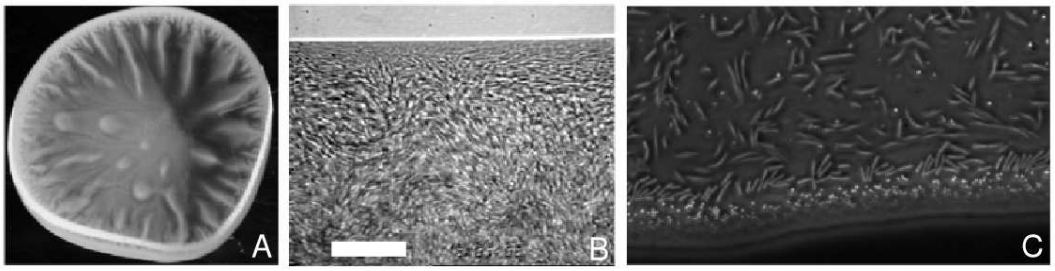

Figure 4. Three scales of organization in suspensions of *B. subtilis*. (A) bioconvection in a sessile drop of diameter 1 cm [22]. (B) close-up of collective swimming in the ZBN near the contact line. Blurring arises from very fast swimming speed. Scale bar is 35 μm. (C) even closer view at lower concentration and in a shallow layer, showing accumulation at the contact line and 0.5 μm

fluorescent microspheres used for flow tracers.

In the self-concentrated region, where the volume fraction exceeds 0.1, we find recurring, transient large-scale swirls (Fig. 4B). These flows are analyzed with particle-imaging-velocimetry, PIV, where the bacteria act both as flow generators and markers. Such measurements are projections onto the in-plane dimensions $\mathbf{x}_\parallel = (x, y)$ of three-dimensional patterns. Figure 4 (left) shows a typical velocity field $\mathbf{v}(\mathbf{x}_\parallel)$, with a meandering *jet* of collective speed ($\sim$ 50 μm/s) and surrounding *vortices*. The vortices nearest the leftward-directed jet flow circulate clockwise above and counterclockwise below, as in a Kelvin-Helmholtz instability or, anomalously at such low Reynolds numbers, a von Kármán vortex street. Coarse-grained image intensity is the basis of this PIV analysis. Measurements using passive tracers yield velocity bursts > 100 μm/s. We have found a second-order phase transition to the ZBN state at a critical concentration, where the number density of such transient structures serves as the order parameter. It should be emphasized that microscopic explanations of these phenomena and for the persistent codirectional alignment of cells are only partially understood [42, 48] and constitute the focus of current investigation.

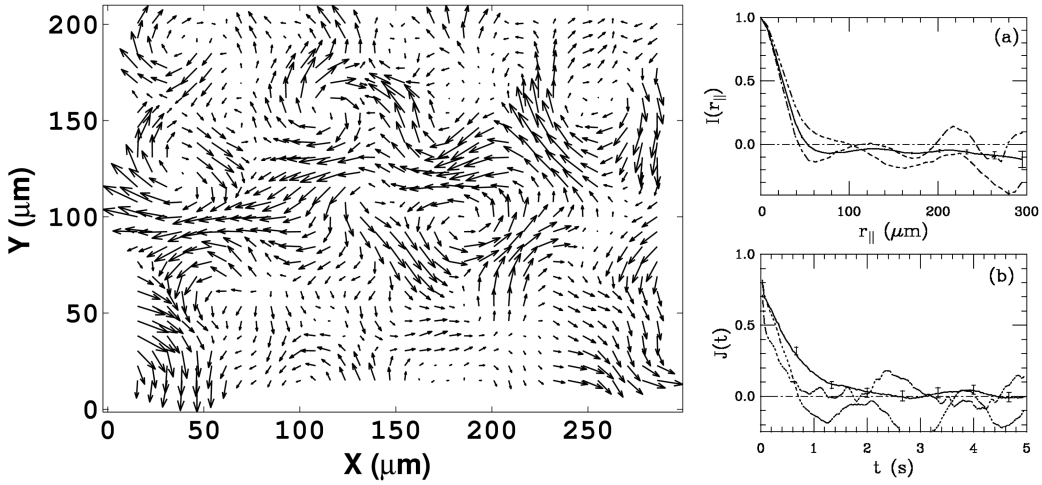

Figure 5. Results from PIV studies of collective behavior in bacterial suspensions [22]. Left: vector field of swimming patterns, and correlation functions in space (a) and time (b).

The velocity correlation $I(\mathbf{r}_\parallel) = (\langle \mathbf{v}(\mathbf{x}_\parallel + \mathbf{r}_\parallel, t) \cdot \mathbf{v}(\mathbf{x}_\parallel, t) \rangle_{\mathbf{x}} - \langle \mathbf{v} \rangle_{\mathbf{x}}^2)/(\langle \mathbf{v}^2 \rangle_{\mathbf{x}} - \langle \mathbf{v} \rangle_{\mathbf{x}}^2)$ measures the persistence of a given swimming direction as a function of distance, at a given time. If we average it over orientations of $\mathbf{r}_\parallel$ it provides a purely radial measure of the spatial decay, denoted $I(r_\parallel)$ and shown in Fig. 5(a) for patterns in a pendant drop. A partner to this quantity is the temporal correlation for that same arrangement, measuring how the velocity decays with time at a given point in space, $J(t) = (\langle \mathbf{v}(\mathbf{x}_\parallel, s+t) \cdot \mathbf{v}(\mathbf{x}_\parallel, s) \rangle_s - \langle \mathbf{v} \rangle_s^2)/(\langle \mathbf{v}^2 \rangle_s - \langle \mathbf{v} \rangle_s^2)$ in Fig. 5(b). Solid lines show data averaged over 500 images (16.7 s). Correlations from several individual pairs of frames are superimposed. The latter display quite pronounced oscillations reflecting the particular positions of the vortices. The much smoother average clearly shows anticorrelation extending out to ~ 100 μm, defining the typical scale of a vortex, and coherence lasting a few seconds, the "natural" time scale $\tau = \langle \text{domain size} \rangle / \langle \text{domain speed} \rangle$. The average correlation in Fig. 5(b) implies that

a directional surge is followed by a return flow; the two particular cases demonstrate vortex street generation. The spontaneous development of dynamic long range coherence, persistent yet intermittent, produces mixing and transport of metabolites, wastes and signaling molecules well beyond diffusion. For typical correlation lengths and velocities, even for small molecules such as O_2, the Peclet number is of order 10. Intermittency, changes of coherent velocity direction and collision of domains implies chaotic transport and mixing beyond simple advection. The observation that the decay interval of coherent domains tends approximately to match the time interval ($\sim$ 1 s) required for consuming "all" the dissolved oxygen by a close-packed population of *B.subtilis* is strong circumstantial evidence correlating metabolic requirements, transport and dynamic coherence.

Evidently, great populations of single swimming cells, using hydrodynamic interactions to accomplish jointly the tasks necessary for survival, have a functional commonality with the permanently joined primitive flagellated organisms at the periphery of volvocalean colonies. Is there perhaps an analogous evolved design of these bacteria for coupling the dynamic of these organisms with the physical dynamics of their aqueous surround? Does evolution arrive at motile cells that singly can seek favorable environments, yet act efficiently and jointly, once an environment has been found that permits the multiplication of a few explorers into vast populations? Perhaps our research provides insights, or at least permits the development of sharper questions [49].

5 Further Discussion

We have seen two biological examples in which relatively high-speed fluid flows with long-range correlations are produced by the action of multiple individual flagella, resulting in enormously enhanced transport relative to diffusion. The volvocalean algae provide an example in which those flows are generally quite regular; bacterial collective behavior is much more stochastic. Yet, in each case the transport of molecular nutrients or chemical messengers to and from remote regions is greatly enhanced relative to the rate possible from an isolated individual. It therefore appears likely that these cooperative effects could have played an important role in the transition from single-cell organisms to multicellular ones.

We find that loose aggregations of bacterial cells with no *apparent* specialization can generate cooperative behaviors acting as a coherent multicellular group. This association is transient and flexible and, depending on environmental conditions, this association may or may not form.

On the other hand, when cell associations are permanent as in actual multicellular organisms, selective pressures played a major role. The first result, a tendency for multicellular organisms to increase in size, entailed the costs of reproducing an increasingly larger group, with increasingly negative effects on their viability. Beyond definite threshold sizes, the fitness of the multicellular organism decreased unless it invested in cell specialization (i.e., germ-soma separation). That resulted in the evolution of new levels of individuality and the emergence of higher level units. We can infer that increased complexity and individuality can be a consequence of trade-offs between the two basic fitness components – fecundity (i.e. investment in reproductive tissue) and viability (i.e. investment in sterile flagellated tissue for motility and nutrient acquisition) as size increases. The limited resources available to these organisms have to be partitioned to optimize fitness. As multicellular individuals or groups increase in size, the *allocation strategy* changes to optimize the fitness of the organisms: In volvocalean colonies, undifferentiated cells perform both motility and reproductive functions. In larger colonies, more resources must be allocated to

motility, to avoid sinking and increase nutrient acquisition. The organism then invests in somatic cells that are permanently flagellated and do not perform reproductive functions. The higher costs of reproducing a larger organism and the trade-offs between reproducing, surviving, and acquiring resources generate the transition to higher levels of organization and complexity. Each degree of specialization and differentiation can counteract the higher costs associated with larger size, by increasing the viability and/or the productivity (fecundity) of the enlarged organism, allowing it to reach fitness levels impossible to attain without increased complexity.

6 Conclusion

Our results on bacterial and algal systems demonstrate that transitions from uni- to multicellular organisms necessarily take place in the context of their interactions with a fluid environment. For both of these model biological systems the balance between the effects of stirring and diffusion, summarized by the Peclet number, switches from diffusion-dominated behavior for single cells to stirring-dominated for the multicellular species. Associated with this can be nonlinearly-enhanced rates of nutrient uptake and waste removal, key components of the fitness of organisms. These can in fact provide evolutionary driving forces for organisms to increase in size. Also, the genetic relationship between the species displaying germ-soma separation and the totipotent lower species among the Volvocales highlights an important evolutionary feature: the appearance of new behaviors need not come from fundamentally new genetic programs, but rather switches which turn on and off pre-existing capabilities. Finally, at least for the colonial algae, there are strong couplings between the developmental changes associated with reproductive modes in the higher species and the very flagella which provide the stirring. Our perspective reveals that the evolution of multicellularity and cellular specialization can just be described as the evolution of novel life-history traits as a means of dealing with the fitness trade-offs of survival and reproduction under the selective pressure of forming increasingly larger adult groups.

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