Dance of the microswimmers

Eric Lauga and
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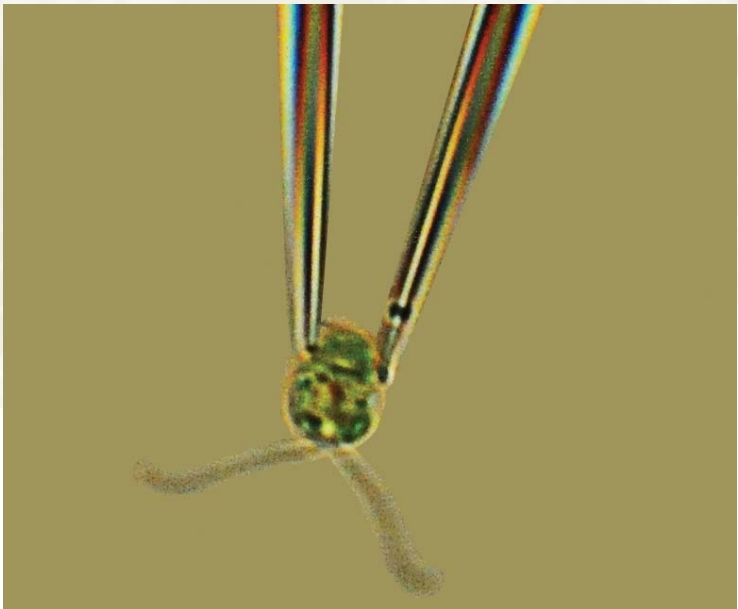

From gut-inhabiting bacteria to sea-dwelling algae, microorganisms display a penchant for coordinated movement. Nonlinear dynamics and fluid mechanics can help explain the curious behavior.

In his long and astonishingly productive life, Dutch scientist Antoni van Leeuwenhoek not only made fundamental and far-reaching technical improvements to the microscope, he also discovered a remarkable variety of microorganisms that are now central to much of biology. In a 1676 letter to the Royal Society of London, he described his initial glimpses of tiny creatures he termed animalcules, which we now know as bacteria:

> I now saw very distinctly that these were little eels or worms . . . lying huddled together and wriggling, just as if you saw with your naked eye a whole tubful of very little eels and water, the eels moving about in swarms; and the whole water seemed to be alive with the multitudinous animalcules. For me this was among all the marvels that I have discovered in nature the most marvelous of all, and I must say that, for my part, no more pleasant sight has yet met my eye than this of so many thousands of living creatures in one small drop of water, all huddling and moving, but each creature having its own motion.

Here, van Leeuwenhoek points to two interrelated aspects of microbial locomotion—the individual dynamics of single, swimming cells and the collective motions that arise when many of those cells interact. Over the past decade, exciting theoretical and experimental discoveries have converged to elevate those two topics into the forefront of biophysics research. The fascination of the fast-growing field derives in part from the way it couples hydrodynamics to areas ranging from nonlinear and statistical physics to cell biology, biotechnology, and applied mathematics. But much of the appeal remains just as it was for van Leeuwenhoek: the mesmerizing dance of the microswimmers.

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Doing the locomotion

Life as we know it can be divided into two groups: eukaryotes, which have cellular nuclei, and prokaryotes—bacteria and archaea—which don't. In turn, swimming prokaryotic and eukaryotic microbes can be distinguished by the nature of their flagella, the hair-like cellular appendages that enable locomotion. Prokaryotic flagella, including those found on the bacterium *Escherichia coli*, are rigid, helical, and passive. A rotary motor embedded in the cell wall rotates the flagellum at its point of attachment, and that rotation propels the cell forward. (See the article by Howard Berg, PHYSICS TODAY, January 2000, page 24.) The effect is not unlike a corkscrew pulling itself through the cork of a wine bottle, except that the flagellum advances less than a full wavelength per turn.

In contrast, eukaryotic flagella, such as those deployed by spermatozoa and green algae, are flex-

ible and actively deforming. Molecular motors distributed along the flagellum's length produce bending moments, and the coordinated action of those motors generates a wave-like undulatory motion. The wave's frequency can range from a few to a hundred hertz, and its shape can take one of two forms. The so-called flagellar waveform resembles a sinusoidal traveling wave; it pushes fluid in the direction of the wave propagation and the microorganism in the other.

When several eukaryotic flagella are closely spaced on a surface to form a carpet, they are often referred to as cilia. An individual cilium's waveform is an asymmetric cycle, with an extended power stroke in one direction followed by a compact recovery stroke; the result is a net transport of fluid along the cell surface.[1]

Flagella and cilia are among the most highly conserved structures in biology: the eukaryotic flagella that first appeared on Earth in single-cell organisms some billion years ago are essentially identical to the cilia within humans, the most highly developed eukaryotes. The ability to manipulate fluid and to maneuver within it is important to nearly every form of life. Cilia, in particular, are essential to human development and physiology: They help to clear mucus from our respiratory system, waft ova along the female reproductive tract, and establish the left–right asymmetry of the developing vertebrate embryo. Defective cilia underlie a number of human diseases; many of them can be studied in the lab using flagellated single-cell organisms as proxies.

Getting in step

Flagella don't all beat to the same drum. Each has a frequency that depends on the number and detailed properties of its molecular motors and on the biochemical signals it receives from within the cell and the nearby environment. And those signals are subject to noise, the inevitable fluctuation of local chemical concentrations. Nevertheless, when flagella happen to come within a few microns of each other, they tend to do something that on its face seems improbable: They move in synchrony. Examples of that complex, nonlinear behavior are illustrated in figure 1.

In figure 1a, two mammalian spermatozoa, each propelled by a single eukaryotic flagellum, approach one another, and their flagella lock phases. The cells can swim together in phase for many beating cycles—up to a few tens of periods for human spermatozoa—shadowing each other so closely as to appear indistinguishable. Similar phase-locking behavior has been seen for as many as four cells at a time.[2] On occasion, spermatozoa have been seen to synchronize in antiphase, with the cells and their flagella moving as mirror images.

Figure 1b shows a single eukaryotic cell—*Chlamydomonas reinhardtii*, a freshwater alga—that propels itself by doing a breaststroke: Its two flagella alternately draw away from and toward each other, moving in synchrony for up to thousands of periods at a time. From time to time, however, biochemical noise causes the cell to temporarily desynchronize.[3]

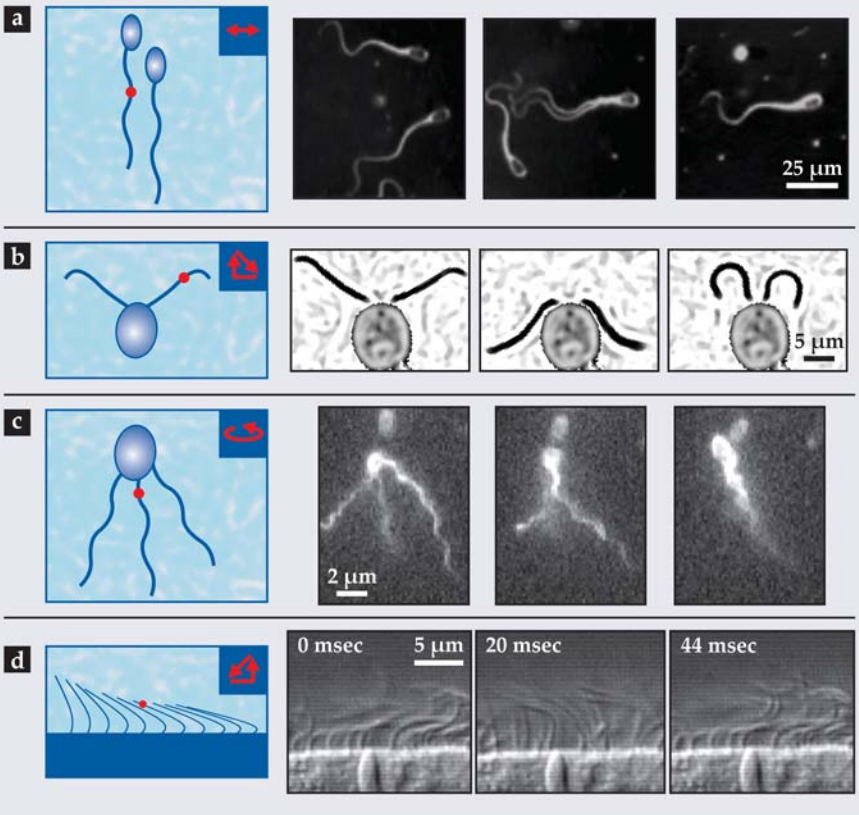

Figure 1. Synchronized swimming. **(a)** The flagellum of a free-swimming spermatozoon deforms in a traveling, transverse wave, as illustrated in the sketch at left. (For each sketch, the inset red arrows show the motion of the flagellum at the point indicated by the dot.) When two spermatozoa approach each other, their flagella attract and move in synchrony. (Adapted from ref. 2.) **(b)** An alga cell pulls itself along by doing a sort of breaststroke: Its two flagella draw away from each other with a power stroke and then draw toward each other with a recovery stroke. (Adapted from ref. 3.) **(c)** A bacterium's rigid, helical flagella rotate like corkscrews to propel the cell forward. The images at right show the flagella of an *Escherichia coli* bacterium attracting and synchronizing in a coherent bundle. (Adapted from ref. 4.) **(d)** Cilia, flagella that line a cell surface to form a carpet, move with a power stroke in one direction followed by a recovery stroke in the other. At right, they are shown in various stages of a coordinated motion known as a metachronal wave. (Adapted from ref. 15.)

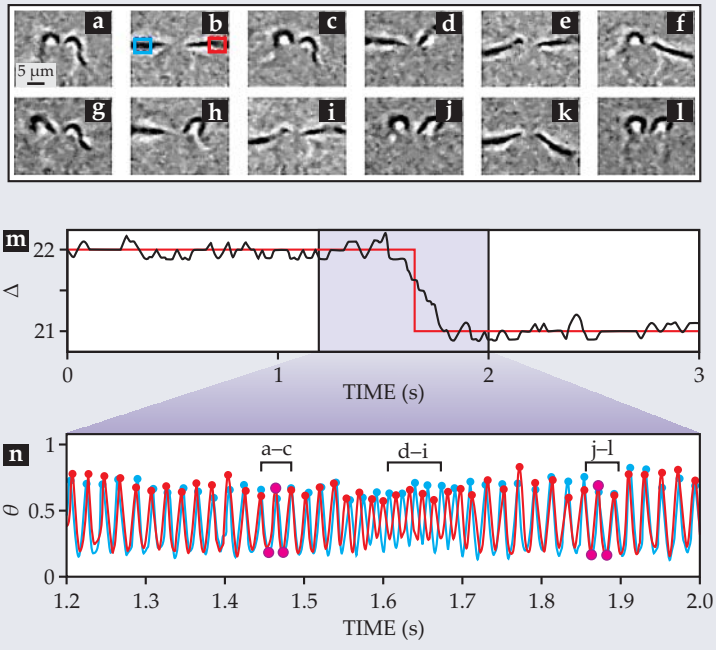

Figure 2. Phase slip. The two flagella that emerge from the alga *Chlamydomonas reinhardtii* usually mirror each other with near-perfect synchrony. But in an occurrence known as a phase slip, the flagella transition from a phase-locked state **(a–c)** to an asynchronous state **(d–i)** and into a new phase-locked state **(j–l)**. Time plots of experimental data **(m,n)** show that the phase slip, which occurs in just a fraction of a second, manifests as an integer jump in the flagella's relative phase $\Delta = (\theta_1 - \theta_2)/2\pi$, where θ_1 and θ_2 are the phases of the points indicated by the blue and red boxes in panel b. (Adapted from ref. 3.)

The ancestors to the eukaryotes, the prokaryotes, also synchronize their flagella, as is the case for *E. coli* (see figure 1c). A typical *E. coli* cell is randomly appended with up to 10 or so helical flagella, each independently driven by its own rotary motor. Even so, they tend to attract and phase lock, forming a coherently rotating bundle. Every now and then, the bundle briefly splays open and reforms, allowing the bacterium to change direction and explore its environment.[4]

Eukaryotic cilia are unique in that they typically synchronize with a phase gradient in one direction along the cell or tissue surface from which they protrude (see figure 1d). The result is a coherent collective deformation known as a metachronal wave, which resembles the wave performed by fans in sports stadiums.

The above examples demonstrate the breadth of geometries and the wide variety of flagella types for which synchronization can occur. Researchers have known about most of those synchronization phenomena for decades and have been measuring the dynamics of undulatory swimming since James Gray's pioneering work on locomotion in the 1930s. But only recently have we developed the capability to quantify phase dynamics in experiments. It is now possible, for instance, to track an entire flagellar waveform throughout its full beating cycle or, more simply, to track the orbit of a single point along the flagellum. The latter suffices to generate a measure of the time-dependent phase angle $\theta(t)$. The group of one of us (Goldstein) used that strategy to study phase locking between the paired flagella of a *Chlamydomonas* cell.[3] The dynamic state of the flagella can be represented as a relative phase difference, $\Delta = (\theta_1 - \theta_2)/2\pi$, where θ_1 and θ_2 are the phases of the individual flagella (see figure 2). Although Δ always fluctuates due to noise, a Δ that hovers closely to an integer value is an indication that the two flagella are in phase.

In the experiment, the two flagella were observed to lock phases for many beating periods before exhibiting what's known as a phase slip: In an instant spanning about 10 periods, the flagella would fall out of sync and then resynchronize in a new phase-locked state, with Δ having shifted by an integer. Similar phase slips occur in all kinds of nonlinear oscillators, including a variety of classical and quantum condensed-matter systems, and in biological contexts as diverse as circadian rhythms and biochemical oscillators.

Deciphering the dance

Why does phase locking—and sometimes phase slip—occur? Do cells instinctively "decide," via biochemical signaling, when and how to coordinate their own movements, or is a simpler physical mechanism at play? There are no definitive answers yet to those questions, but an emerging consensus is that hydrodynamic interactions, not biochemical signaling, provide the fundamental coupling that leads to stable phase locking.

When a flagellum moves, it pushes and pulls its surrounding fluid, which, in turn, exerts a stress on other flagella. If the fluid appears very viscous on the scale of the microswimmers—that is, if the Reynolds number *Re* is small—those hydrodynamic forces can extend over long distances. By definition, *Re* is UL/ν, the product of the swimmer's velocity and characteristic length scale divided by the fluid's kinematic viscosity. So at the tiny length scales and speeds relevant to microswimmers, *Re* is almost always nearly zero. As a result, hydrodynamic interactions between microswimmers are long ranged, decaying as $1/r$ near flagella, $1/r^2$ far from cells, and $1/r^3$ near solid surfaces. The implication is that any two flagella, near or far, exert a nontrivial hydrodynamic stress on each other. Flagellar synchronization therefore sits squarely within a broad class of problems involving noisy nonlinear oscillators with long-range coupling, a subject with a long history in the mathematical sciences and with applications ranging from pendulum clocks to pacemaker cells in the heart.[5]

From the hydrodynamic perspective emerges a tidy physical picture of flagellar dynamics: At any instant, the forces driving the beating of a flagellum must balance with the viscous drag of the surrounding fluid, the long-range hydrodynamics stresses exerted by neighboring flagella, and the flagellum's own internal resistance to bending and twisting. Perhaps the simplest implementation of that model, proposed in 1951 by G. I. Taylor,[6] treats a pair of flagella as two infinitely wide, waving sheets, each of which deforms according to a traveling sinusoidal

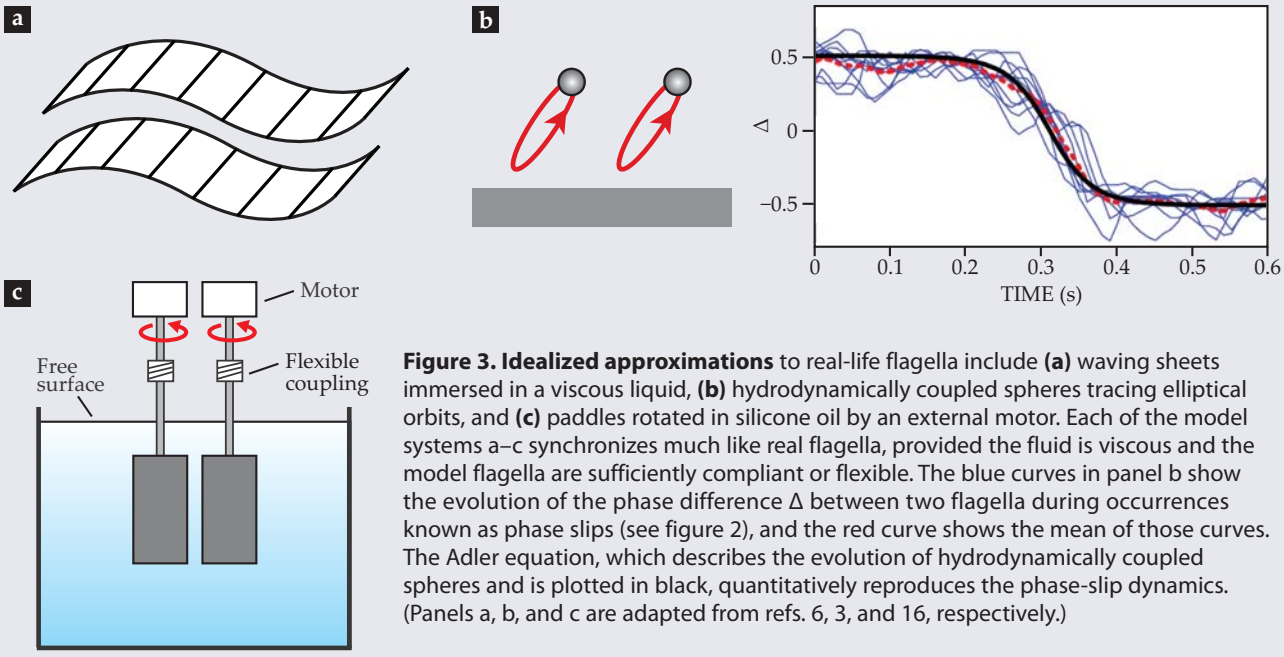

Figure 3. Idealized approximations to real-life flagella include **(a)** waving sheets immersed in a viscous liquid, **(b)** hydrodynamically coupled spheres tracing elliptical orbits, and **(c)** paddles rotated in silicone oil by an external motor. Each of the model systems a–c synchronizes much like real flagella, provided the fluid is viscous and the model flagella are sufficiently compliant or flexible. The blue curves in panel b show the evolution of the phase difference Δ between two flagella during occurrences known as phase slips (see figure 2), and the red curve shows the mean of those curves. The Adler equation, which describes the evolution of hydrodynamically coupled spheres and is plotted in black, quantitatively reproduces the phase-slip dynamics. (Panels a, b, and c are adapted from refs. 6, 3, and 16, respectively.)

wave (see figure 3a). Taylor's calculations for the $Re = 0$ case show that the most energetically favorable scenario is the one in which the waves travel in phase. That result provides a rationale for why phase locking might occur, but technically, it doesn't address the question of whether flagella should transition from any arbitrary state to a phase-locked one. Recent work has shown that if Taylor's model is modified to allow compliance—that is, if each waving sheet is allowed to deviate elastically from its prescribed waveform as if connected to it by a spring—then the sheets lock phases regardless of their initial conditions.

Another approach—a near-literal take on what has come to be known as a spherical-cow model—is to represent flagella and cilia not as sheets but as hydrodynamically interacting spheres.[7] Each sphere is elastically constrained to an elliptical trajectory, in similar fashion to the waving-sheet model (see figure 3b). Just like more realistic descriptions of flagella waveforms, the model yields a nonlinear dynamical equation for the phase difference Δ that, in the simplest case, takes the form $\dot{\Delta} = \delta\nu - \varepsilon \sin(2\pi\Delta) + \xi(t)$. Here, $\delta\nu$ is the difference between the intrinsic frequencies of the spheres' orbits; ε is a coupling constant that depends on the fluid viscosity and the elasticity and dimensions of the spheres' elliptical orbits; and ξ is a stochastic noise term that represents biochemical noise.

Absent the noise term, the model is known as the Adler equation, a rather universal model for coupled phase oscillators. With noise, Δ evolves identically to the position of a diffusive, Brownian particle on a tilted washboard potential: Just as the Brownian particle fluctuates for extended time periods near the bottom of a potential energy well and occasionally jumps from one well to the next, the spherical oscillators synchronize for extended periods at a particular integer value of Δ before undergoing the occasional phase slip, in which Δ shifts by one. The phase-slip behavior closely matches that of real flagella. In fact, even the noiseless Adler equation quantitatively reproduces the mean dynamics observed for *Chlamydomonas*. The spherical-oscillator approach can be extended, by coupling numerous spheres in a single system, to model cilia and to predict the conditions that give rise to metachronal waves.[7]

In experiments, a pair of rigid macroscopic paddles rotating in a viscous fluid can serve as a stand-in for prokaryotic flagella (see figure 3c). Studies show that if each paddle is rigidly connected to its driving motor, the paddles won't lock phases—even if they are driven by identical or near-identical driving torques. If, however, the paddles are connected to their motors via compliant shafts, they synchronize within a few tens of periods. Theoretical models

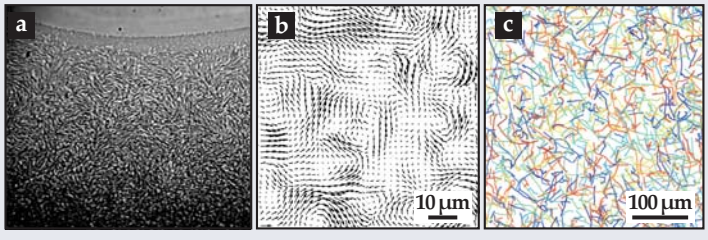

Figure 4. Suspensions of swimming bacteria exhibit transient, recurring states of collective motion known as bacterial turbulence. **(a)** In the turbulent state, densely suspended *Bacillus subtilis* microbes adopt local, but not long-range, orientational order. **(b)** A snapshot of their instantaneous velocities shows a pattern of vortices and jets. **(c)** For a suspension of *Escherichia coli*, a map of swimmers' trajectories over an eight-second period reflects the chaos and disorder that prevails at longer time scales. (Panels a and b are adapted from ref. 10; panel c is adapted from ref. 17.)

suggest that compliance plays a similar role in the synchronization of rotating helical filaments.[8]

The simple, low-dimensional models described above paint a consistent picture in which the essential ingredients for synchronization are twofold: Flagella must interact via long-range hydrodynamic forces, and they must be either flexible or flexibly coupled to their driving mechanism. Yet a number of important issues are still unresolved.[9] Other mechanisms, including biochemical coupling, could potentially also lead to synchronization, and their importance remains to be quantified. Also, the factors that determine whether flagella synchronize in antiphase, as is the case for *Chlamydomonas*, or in phase, as is the case for cilia and most spermatozoa, aren't well known. Those distinct behaviors could be signatures of more fundamental biological differences.

Although simple models elucidate the qualitative, physical picture of synchronization, a predictive and quantitative approach for the complex three-dimensional beating of flagella is still lacking. And despite extensive study of the collective dynamics of cilia, the specific conditions that give rise to coherent metachronal waves remain unknown. Our hope is that modern microscopy and imaging techniques will produce novel insights into phase dynamics and inspire a new class of models that allow quantitative comparisons between theory and experiments.

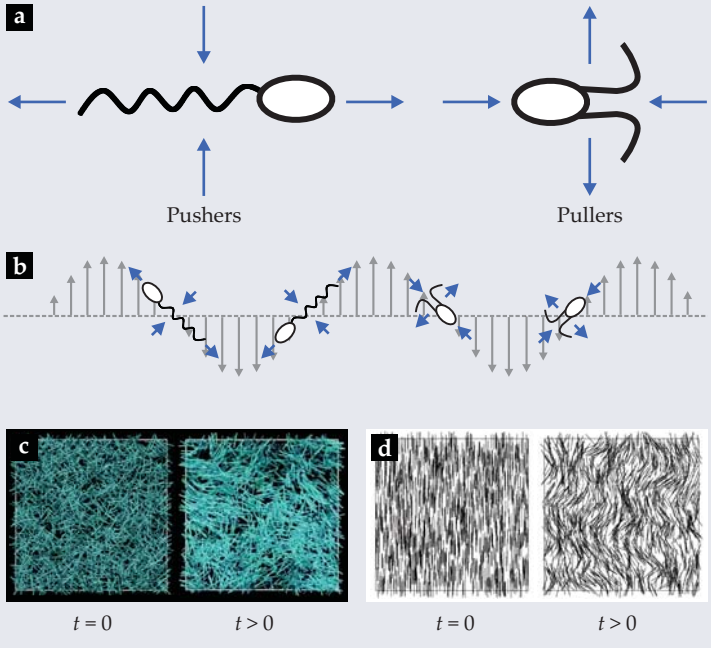

Figure 5. Modeling instability. (a) Pushers, which swim body first, repel fluid in the swimming direction and draw in fluid in the lateral direction. Pullers swim flagella first and create the opposite flow field. (Both of the swimmers sketched here move from left to right.) **(b)** Theory predicts that if a quiescent suspension is perturbed by a sinusoidal flow field, indicated here by the gray arrows, microbes will tend to orient along the principal axis of strain. For pushers, the reorientation causes the swimmer-induced flow field (blue arrows) to amplify the perturbation. For pullers, the swimmer-induced flow field counteracts the perturbation. **(c)** A simulation of rod-shaped pushers shows that an isotropic suspension tends to develop inhomogeneity and local order. **(d)** Simulations also confirm that a suspension of coaligned pushers is unstable. (Adapted from ref. 13.)

A crowded ballroom

Just as multiple flagella often move in concert, groups of swimming cells also display striking collective effects. Indeed, experiments and theory demonstrate qualitative differences between the way a cell swims in isolation and the way it swims as part of a densely populated suspension. For one, dense suspensions of microswimmers have been seen to show transient, recurring formation of swirling vortices and jets whose length scales are large compared with the cell[10] (see figure 4). That state, first described by John Kessler and coworkers, is often referred to as bacterial turbulence. Purists may object to the use of the word turbulence to describe flows at such low *Re*, but it is worth noting that the word's Latin root, *turba*, refers to the disorderly motion of a crowd.

Quantitative measurements show that in a suspension, cells' orientations and velocities tend to correlate on length scales much larger than the cell, typically tens to hundreds of microns. Furthermore, the cells attain much higher velocities than they could in isolation, and the cells may be inhomogeneously distributed in space. The result is an enhancement in the diffusivity of both the cells and the surrounding fluid.[11] Those distinct traits have important implications for understanding bacterial infections, reproductive medicine, and tissue rheology.

Theoretical approaches to understanding collective swimming have followed two paths. The first, microscopic modeling, rests on the fundamental hypothesis that long-range hydrodynamic interactions are responsible for the collective swimming effects we see. That feature distinguishes the collective swimming of microswimmers from high-*Re* phenomena such as the flocking of birds or the schooling of fish.

The microscopic models start with the dynamics of a single organism and then model a large population of those organisms as a dynamical system—the basic physics rests in understanding the fundamental swimmer–swimmer interactions. Each swimmer's properties are typically taken as the average over all of its phases, so a time-averaged interaction between two swimmers is approximated as an interaction between two time-averaged swimmers.

In microscopic models, each swimmer produces a flow field that depends on the nature of its swimming motion. Pushers, including most spermatozoa and bacteria, swim body first, propelled by trailing flagella. They generate a dipolar flow field like the one illustrated in figure 5a, wherein fluid is repelled at the head and tail and drawn in at the sides. In contrast, pullers such as *Chlamydomonas* swim flagella first and generate the opposite flow field.

In parallel with the microscopic modeling, a second approach, coarse-grained modeling, has been used to derive continuum equations for the spatial distribution, velocity, and orientation of microswimmers in suspensions. Some of those models bypass the derivation of specific coefficients and instead use symmetry arguments à la Landau to arrive at very

general equations; other models explicitly derive terms to account for nonlocal swimmer–swimmer interactions, but they apply only in the dilute limit.[12]

Whence the choreography?

What can the models tell us about collective locomotion? So far, they have mostly been used to derive stability criteria. In particular, continuum dilute models predict that a suspension of aligned microswimmers in a quiescent fluid should be unstable to small perturbations, regardless of the organisms' swimming motion. Alternatively, a suspension of isotropically oriented swimmers in a quiescent fluid is unstable if the swimmers are pushers but stable if they are pullers.

A physical explanation for the behavior was first proposed by Sriram Ramaswamy, and the essential mechanism is a coupling between the velocity perturbations in the underlying flow and the orientations of the swimming cells[12] (see figure 5b). Consider an isotropic suspension whose microswimmers are at rest. If the fluid gets disturbed by a sinusoidal velocity perturbation, then theory says the elongated swimmers should tend to align in the direction in which the fluid is being stretched, the principal axis of strain.

But each swimmer also induces a flow due to its own motion: Pushers create a flow field that amplifies the initial flow perturbation and results in flow and orientational instabilities; pullers create a flow that counteracts the perturbation. Note that the physical picture does not rely on explicit hydrodynamic interactions between the cells; it is an intrinsically dilute mechanism. The model's predictions are borne out by computer simulations of dilute suspensions[13] (see figure 5c).

Simulations show that hydrodynamic coupling between swimmers can explain several features of collective swimmers, not just instabilities. But can it explain everything? Recent experiments with swimming bacteria hint that long-range hydrodynamic coupling might not be as strong as previously thought.[14] A new physical picture might emerge in which collective effects simply arise due to competition between excluded-volume effects and noise. Future work—especially detailed experimental investigations—will be needed to fully unravel the physics underlying the collective dance of the microswimmers.

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