Cell-body rocking is a dominant mechanism for flagellar synchronization in a swimming alga

Veikko F. Geyer\textsuperscript{a}, Frank Jülicher\textsuperscript{b}, Jonathon Howard\textsuperscript{a}, and Benjamin M. Friedrich\textsuperscript{b,1}

\textsuperscript{a}Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany; and \textsuperscript{b}Max Planck Institute for the Physics of Complex Systems, 01187 Dresden, Germany

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The unicellular green alga \textit{Chlamydomonas} swims with two flagella that can synchronize their beat. Synchronized beating is required to swim both fast and straight. A long-standing hypothesis proposes that synchronization of flagella results from hydrodynamic coupling, but the details are not understood. Here, we present realistic hydrodynamic computations and high-speed tracking experiments of swimming cells that show how a perturbation from the synchronized state causes rotational motion of the cell body. This rotation feeds back on the flagellar dynamics via hydrodynamic friction forces and rapidly restores the synchronized state in our theory. We calculate that this “cell-body rocking” provides the dominant contribution to synchronization in swimming cells, whereas direct hydrodynamic interactions between the flagella contribute negligibly. We experimentally confirmed the two-way coupling between flagellar beating and cell-body rocking predicted by our theory.

flagellar force–velocity relation | low-Reynolds-number hydrodynamics

Eukaryotic cilia and flagella are long, slender cell appendages that can bend rhythmically and thus present a prime example of a biological oscillator (1). The flagellar beat is driven by the collective action of dynein molecular motors, which are distributed along the length of the flagellum. The beat of flagella, with typical frequencies ranging from 20–60 Hz, pumps fluids, for example, mucus in mammalian airways (2), and propels unicellular microswimmers such as \textit{Paramecia}, spermatozoa, and algae (3). The coordinated beating of collections of flagella is important for efficient fluid transport (2, 4, 5) and fast swimming (6). This coordinated beating represents a striking example for the synchronization of oscillators, prompting the question of how flagella couple their beat. Identifying the specific mechanism of synchronization can be difficult because synchronization may occur even for weak coupling (7). Further, the effect of the coupling is difficult to detect once the synchronized state has been reached.

Hydrodynamic forces were suggested to play a significant role for flagellar synchronization already in 1951 by Taylor (8). Since then, direct hydrodynamic interactions between flagella were studied theoretically as a possible mechanism for flagellar synchronization (9–12). Another synchronization mechanism that is independent of hydrodynamic interactions was recently described in the context of a minimal model swimmer (13–15). This mechanism crucially relies on the interplay of swimming motion and flagellar beating.

Here, we address the hydrodynamic coupling between the two flagella in a model organism for flagellar coordination (16–19), the unicellular green alga \textit{Chlamydomonas reinhardtii}. \textit{Chlamydomonas} propels its ellipsoidal cell body, which has typical diameter of 10 μm, using a pair of flagella, whose lengths are about 10 μm (16). The two flagella beat approximately in a common plane, which is collinear with the long axis of the cell body. In that plane, the two beat patterns are nearly mirror-symmetric with respect to this long axis. The beating of the two flagella of \textit{Chlamydomonas} can synchronize, that is, adopt a common beat frequency and a fixed phase relationship (16–19). In-phase synchronization of the two flagella is required for swimming along a straight path (19). The specific mechanism leading to flagellar synchrony is unclear.

Here, we use a combination of realistic hydrodynamic computations and high-speed tracking experiments to reveal the nature of the hydrodynamic coupling between the two flagella of free-swimming \textit{Chlamydomonas} cells. Previous hydrodynamic computations for \textit{Chlamydomonas} used either resistive force theory (20, 21), which does not account for hydrodynamic interactions between the two flagella, or computationally intensive finite element methods (22). We employ an alternative approach and represent the geometry of a \textit{Chlamydomonas} cell by spherical shape primitives, which provides a computationally convenient method that fully accounts for hydrodynamic interactions between different parts of the cell. Our theory characterizes flagellar swimming and synchronization by a minimal set of effective degrees of freedom. The corresponding equation of motion follows naturally from the framework of Lagrangian mechanics, which was used previously to describe synchronization in a minimal model swimmer (13, 15). These equations of motion embody the key assumption that the flagellar beat speeds up or slows down according to the hydrodynamic friction forces acting on the flagellum, that is, if there is more friction and therefore higher hydrodynamic load, then the beat will slow down. This assumption is supported by previous experiments that showed that the flagellar beat frequency decreases when the viscosity of the surrounding fluid is increased (23, 24). The simple force–velocity relationship for the flagellar beat employed by us coarse-grains the behavior of thousands of dynein molecular motors that collectively drive the beat. Similar force–velocity properties have been described for individual molecular motors (25) and reflect a typical behavior of active force generating systems.

Our theory predicts that any perturbation of synchronized beating results in a significant yawing motion of the cell, reminiscent of rocking of the cell body. This rotational motion imparts different hydrodynamic forces on the two flagella, causing one of them to beat faster and the other to slow down. This interplay

Significance

The eukaryotic flagellum is a best-seller of nature: These slender cell appendages propel sperm and many other microswimmers, including disease-causing protists. In mammalian airways or the oviduct, collections of flagella beat in synchrony to pump fluids efficiently. Flagellar synchronization was proposed to rely on mechanical feedback by hydrodynamic forces, but the details are not well understood. Here, we used theory and experiment to elucidate a mechanism of synchronization in the model organism \textit{Chlamydomonas}, a green algal cell that swims with two flagella like a breaststroke swimmer. Our analysis shows how synchronization arises by a coupling of swimming and flagellar beating and characterizes an exemplary force–velocity relationship of the flagellar beat.

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1To whom correspondence should be addressed. E-mail: benjamin.friedrich@pks.mpg.de.

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between flagellar beating and cell-body rocking rapidly restores flagellar synchrony after a perturbation. Using the framework provided by our theory, we analyze high-speed tracking experiments of swimming cells, confirming the proposed two-way coupling between flagellar beating and cell-body rocking.

Previous experiments restrained *Chlamydomonas* cells from swimming, holding their cell body in a micropipette (17–19). Remarkably, flagellar synchronization was observed also for these constrained cells. This observation seems to argue against a synchronization mechanism that relies on swimming motion. However, the rate of synchronization observed in these experiments was faster by an order of magnitude than the rate we predict for synchronization by direct hydrodynamic interactions between the two flagella in the absence of any motion. In contrast, we show that the oscillation motion with a small amplitude of a few degrees only, which may result from either a residual rotational compliance of the clamped cell or an elastic anchorage of the flagellar pair, provides a possible mechanism for rapid synchronization, which is analogous to synchronization by cell-body rocking in free-swimming cells.

**Results and Discussion**

**High-Precision Tracking of Confined Chlamydomonas Cells.** To study the interplay of flagellar beating and swimming motion, we recorded single wild-type *C. reinhardtii* cells swimming in a shallow observing chamber using high-speed phase-contrast microscopy (1,000 frames per second). The chamber heights were only slightly larger than the cell diameter so that the cells did not roll around their long body axis, but only translated and rotated in the focal plane. This confinement of cell motion to two space dimensions and the fact that the approximately planar flagellar beat was parallel to the plane of observation greatly facilitated data acquisition and analysis. From high-speed recordings we obtained the projected position and orientation of the cell body as well as the shape of the two flagella (Fig. 1A and Fig. S1).

In the reference frame of the small body, each flagellum undergoes periodic shape changes. To formalize this observation, we defined a phase angle variable by binning flagellar shapes according to shape similarity (Fig. 1B and Fig. S2). A time series of flagellar shapes is represented by a point cloud in an abstract shape space. This point cloud comprises an effectively one-dimensional shape cycle, which reflects the periodicity of the flagellar beat. Each shape point can be projected on the centerline of the point cloud. We define a phase variable \( \phi \) running from 0 to \( 2\pi \) that parameterizes this limit cycle by requiring that the phase speed be constant for synchronized beating. Approximately, we determine this parameterization from the condition that the averaged phase speed is independent of the location along the limit cycle. This defines a unique flagellar phase for each tracked flagellar shape. The width of the point cloud shown in Fig. 1B is a measure for the variability of the flagellar beat during subsequent beat cycles. We find that the variations of flagellar shapes for the same value of the phase variable are much smaller than the shape changes during one beat cycle. For our analysis, we therefore neglect these variations of the flagellar beat. In this way, we characterize a swimming *Chlamydomonas* cell by 5 degrees of freedom: its position \((x, y)\) in the plane, the orientation angle \( \alpha \) of its cell body, and the two flagellar phase variables \( \phi_L \) and \( \phi_R \) for the left and right flagellum, respectively. Our theoretical description will employ the same 5 degrees of freedom to describe flagellar shapes parameterized by a single phase variable \( \phi \).

**Hydrodynamic Forces and Interactions.** For a swimming *Chlamydomonas* cell, inertial forces are negligible [as characterized by a low Reynolds number of \( \text{Re} \sim 10^{-2} \) (22)], which implies that the hydrodynamic friction forces exerted by the cell depend only on its instantaneous motion (26). To conveniently compute hydrodynamic friction forces and hydrodynamic interactions, we represented the geometry of a *Chlamydomonas* cell by 300 spherical shape primitives (Fig. 2A). The spheres constituting the cell body are treated as a rigid cluster. For simplicity, we consider free-swimming cells and do not include wall effects in our hydrodynamic computations. Flagellar beating and swimming corresponds to a simultaneous motion of all 300 spheres of our cell model. The dependence of the corresponding hydrodynamic friction forces and torques on the velocities of the individual spheres is characterized by a grand hydrodynamic friction matrix \( G \). We computed this friction matrix \( G \) using a Cartesian multipole expansion technique (27); *Materials and Methods* gives details. Fig. 2C and D shows a submatrix that relates forces and velocity components parallel to the long axis of the cell. The entries of the color matrix depict the force exerted by any of the flagellar spheres or by the cell-body cluster (row index), if a single flagellar sphere or the cell-body cluster is moved (column index). The indexing of flagellar spheres is indicated by cartoon drawings of the cell next to the color matrix. The diagonal entries of this friction matrix are positive and account for the usual Stokes friction of a single “flagellar sphere” (or of the cell body). Off-diagonal entries are negative and represent hydrodynamic interactions. We find considerable hydrodynamic interactions between spheres of the same flagellum, as well as between each flagellum and the cell body. However, interactions between the two flagella are comparably weak.

**Theoretical Description of Flagellar Beating and Swimming.** We now present dynamical equations for the minimal set of 5 degrees of freedom shown in Fig. 1A to describe flagellar beating, swimming,
and later flagellar synchronization in *Chlamydomonas*. These equations of motion follow naturally from the framework of Lagrangian mechanics of dissipative systems, which defines generalized forces conjugate to effective degrees of freedom.

Motivated by our experiments, we describe the progression through subsequent beat cycles of each of the two flagella by respective phase angles \( \phi_1 \) and \( \phi_2 \) (Fig. 1A). The angular frequency \( \omega_1 \) of flagellar beating is given by the time-averaged phase speed \((\bar{\omega})_1\), so we can think of the phase speed as the instantaneous beat frequency. We are interested in variations of the phase speed that can restore a synchronized state after a perturbation. We introduce the key assumption that changes in hydrodynamic friction during the flagellar beat cycle can increase or decrease the phase speed of each flagellum. Specifically, we assume that for both the left and right flagellum, \( j = L, R \), the respective flagellar phase speed \( \dot{\phi}_j \) is determined by a balance of an active driving force \( Q_j \) that coarse-grains the active processes within the flagellum and a generalized hydrodynamic friction force \( P_j \), which depends on \( \dot{\phi}_j \). Note that in addition to hydrodynamic friction, dissipative processes within the flagella may contribute to the friction forces \( P_L \) and \( P_R \). We do not consider such internal friction in our description because it does not change our results qualitatively. The hydrodynamic friction forces \( P_L \) have to be computed self-consistently for a swimming cell.

We restrict our analysis to planar motion in the "xy" plane and thus consider the position \((x, y)\) and the orientation \( \alpha \) of the cell body with respect to a fixed laboratory frame (Fig. 1A).

Any change of the degrees of freedom \( x, y, \alpha, \dot{\phi}_1, \) or \( \dot{\phi}_2 \) results in the dissipation of energy into the fluid at some rate \( \mathcal{R} \). This dissipation rate \( \mathcal{R} \) characterizes the mechanical power output of the cell and plays the role of a Rayleigh dissipation function known in Lagrangian mechanics; it can be written as \( \mathcal{R} = xP_L + yP_R + \alpha P_L + \dot{\phi}_1 P_L + \dot{\phi}_2 P_R \), which defines the generalized friction forces \( P_L \) and \( P_R \) and \( \dot{\phi}_1 \) and \( \dot{\phi}_2 \) are conjugate to an angle and have physical units, piconewtons times micrometer. We compute the generalized friction forces using the grand hydrodynamic friction matrix \( G \) introduced above. In brief, the superposition principle of low-Reynolds-number hydrodynamics relevant for *Chlamydomonas* swimming (26) implies that the generalized friction forces relate linearly to the generalized velocities, \( P_L = \Gamma_{\alpha} \dot{\alpha} + \Gamma_{\phi_1} \dot{\phi}_1 + \Gamma_{\phi_2} \dot{\phi}_2 \) and \( P_R = \Gamma_{\alpha} \dot{\alpha} + \Gamma_{\phi_1} \dot{\phi}_1 + \Gamma_{\phi_2} \dot{\phi}_2 \). This defines the generalized hydrodynamic friction coefficients \( \Gamma_{ij} \), which are suitable linear combinations of the entries of the grand hydrodynamic friction matrix \( G \) (Materials and Methods and Figs. S3 and S4).

The friction force \( P_L \) conjugate to the \( x \) coordinate of the cell position represents just the \( x \) component of the total force and the cell body and the \( y \) component of the total force \( P_R \) acts on the cell body, and an analogous analysis applies for \( P_R \); \( P_R \) is the total torque associated with rotations around an axis normal to the plane of swimming. If the swimmer is free from external forces and torques, we have \( P_L = P_R = 0 \), because \( P_L = P_R = 0 \). Together with the proposed balance of flagellar friction and driving forces, \( F_L = Q_L \) and \( F_R = Q_R \), we have a total of five force balance equations, which allow us to solve for the time derivatives of the 5 degrees of freedom. We obtain an equation of motion that combines swimming and flagellar phase dynamics

\[
(x, y, \alpha, \dot{\phi}_1, \dot{\phi}_2) = \Gamma^{-1}(0, 0, 0, Q_L, Q_R)^T.
\]

The phase dependence of the active driving forces \( Q_L(\phi_1) \) and \( Q_R(\phi_2) \) is uniquely specified by the condition that the phase speeds should be constant, \( \dot{\phi}_1 = \omega_1 \) for synchronized flagellar beating with zero flagellar phase difference \( \delta = 0 \), where \( \delta = \phi_1 - \phi_2 \).

In essence, this generic description implies that the phase speed of one flagellum is determined by hydrodynamic friction forces, which in turn depend on the swimming motion of the cell. Because the swimming motion is determined by the beating of both flagella, Eq. 1 effectively defines a feedback loop that couples the two flagellar oscillators.

**Theory and Experiment of Chlamydomonas Swimming.**

Using the equation of motion (Eq. 1), we can compute the swimming motion of our model cell. For mirror-symmetric flagellar beating with zero flagellar phase difference \( \delta = 0 \), the model cell follows a straight path with an instantaneous velocity that is positive during the effective stroke but becomes negative during a short period of the recovery stroke (Fig. 3A, Left). *Chlamydomonas* swims two steps forward, one step back. This saltatory motion is also observed experimentally by us (Fig. 3A, Right) and others (16, 28, 29). In our computation, the instantaneous swimming velocity reaches values up to 200 \( \mu m/s \), which agrees with experimental measurements for free-swimming cells (29), but overestimates the observed translational swimming speeds in shallow chambers, in which wall effects are expected to reduce the speed of translational motion (compare left and right panels in Fig. 3A). If the two flagella are beating out of phase, the cell will not swim straight anymore, but the cell body yaws (Fig. 3B). Cell-body yawing is observed experimentally (Fig. 3B, Right), with measured yawing rates that agree well with our computations (Fig. 3B, Left). The proximity of boundary walls is known to reduce translational motion but to affect rotational motion to a much lesser extent for a given distance from the wall (21). This is indeed observed in our experiments with cells swimming in shallow chambers: Whereas the observed translational speed is smaller than predicted (Fig. 3A and Fig. S5), the observed yawing rates are very similar to the

![Image of hydrodynamic interactions between the two flagella](image-url)
flagellum, causing the left flagellum to beat slower and the right one to beat faster. As a result, flagellar synchrony is restored.

Next, we present a formalized version of this argument using a reduced equation of motion. We thus arrive at a simple theory for biflagellar synchronization, which will later allow for quantitative comparison with experiments. As in Fig. 4B, we assume that the cell is constrained such that it cannot translate \((\dot{x} = \dot{y} = 0)\). The cell can still yaw, possibly being subject to an elastic restoring force \(Q_y = -k\alpha\). This leaves only 3 degrees of freedom: \(\dot{\varphi}_L\), \(\dot{\varphi}_R\), and \(\alpha\). Neglecting direct hydrodynamic interactions between the flagella, we can reduce the equations of motion for a clamped cell (Eq. 1 with constraint \(\dot{x} = \dot{y} = 0\)) to a set of three coupled equations for the three remaining degrees of freedom:

\[
\dot{\varphi}_L = \alpha_L - \mu(\varphi_L)\dot{\alpha},
\]

\[
\dot{\varphi}_R = \alpha_R + \mu(\varphi_R)\dot{\alpha},
\]

\[
k\alpha + \rho(\varphi_L, \varphi_R)\dot{\alpha} = -\nu(\dot{\varphi}_L)\dot{\varphi}_L + \nu(\dot{\varphi}_R)\dot{\varphi}_R.
\]

The coupling function \(\mu\) in Eq. 2 characterizes the effect of cell-body yawing on the flagellar beat as detailed below, and \(\nu\) describes how asynchronous flagellar beating results in yawing: \(\nu\) is the hydrodynamic friction coefficient for yawing of the whole cell. The coupling functions \(\mu\), \(\nu\), and \(\rho\) can be computed using our

Theory of Flagellar Synchronization by Cell-Body Yawing. We now demonstrate how yawing of the cell body leads to flagellar synchronization. We first examine the flagellar phase dynamics after a perturbation of in-phase flagellar synchrony. Fig. 4A shows numerical results for a free-swimming cell obtained from solving the equation of motion (Eq. 1). The initial flagellar asynchrony causes a yawing motion of the model cell, which is characterized by periodic changes of the cell’s orientation angle \(\alpha(t)\). The phase difference \(\delta\) between the left and right flagellum decays approximately exponentially as \(\delta(t) \sim \exp(-\lambda t/T)\) with a rate constant \(\lambda\) (measured in beat periods \(T = 2\pi/\omega_0\)) that will serve as a measure of the strength of synchronization.

To mimic experiments in which external forces constrain cell motion, we now consider the idealized case of a cell that cannot translate, while cell-body yawing is constrained by an elastic restoring force \(Q_y = -k\alpha\). Again, the two flagella synchronize in-phase, provided some residual cell-body yawing is allowed (Fig. 4B). In the absence of an elastic restoring force \((k = 0)\), when the model cell cannot translate, but can still freely rotate, its yawing motion and synchronization behavior is very similar to the case of a free-swimming cell that can rotate and translate. For a fully clamped cell body, however, the synchronization strength is strongly attenuated and is solely due to the direct hydrodynamic interactions between the two flagella. In this case of synchronization by hydrodynamic interactions, the time constant for synchronization is decreased approximately 20-fold compared to the case of free swimming. These numerical observations point to a crucial role of cell-body yawing for flagellar synchronization. The underlying mechanism of synchronization can be explained as follows. For in-phase synchronization, the flagellar beat is mirror-symmetric and the cell swims along a straight path. If, however, the left flagellum has a small head-start during the effective stroke, this causes a counter-clockwise rotation of the cell (Fig. 3B). This cell-body yawing increases (decreases) the hydrodynamic friction encountered by the left (right)

predicted ones (Fig. 3B). The good agreement between theory and experiment for the yawing rate supports our hydrodynamic computation as well as our description of flagellar beating using a single phase variable. In the next section, we show that rotational motion is crucial for flagellar synchronization, whereas translational motion is less important.

Fig. 3. (A) For synchronized flagellar beating, we compute saltatory forward swimming with positive instantaneous velocity during effective stroke beating, and a backward motion during the recovery stroke (Left); this behavior is summarized by cartoon drawings (Extreme Right). A typical experimental velocity profile of a Chlamydomonas cell in a shallow observation chamber measured during a cycle of synchronized beating is shown for comparison in the middle panel. (B) Flagellar asynchrony causes cell-body yawing, both in theory and experiment. Shown is the instantaneous rotation rate \(\alpha\) of the cell body in color code as a function of the respective phase of the two flagella. For in-phase synchronized flagellar beating (dashed line), the cell body does not rotate (green). For out-of-phase flagellar beating, however, we find significant cell-body rocking (blue, clockwise; red, counter-clockwise).

Fig. 4. Flagellar synchronization by cell-body yawing. (A) For a free-swimming cell (Top), the equation of motion (Eq. 1) predicts a yawing motion of the cell body characterized by \(\alpha(t)\) if the two flagella are initially out of synchrony (Middle). The flagellar phase difference \(\delta\) is found to decrease with time (Bottom, solid line), approximately following an exponential decay \(\delta(t) \sim \exp(-\lambda t/T)\) (dotted line), where \(T\) is the period of the flagellar beat and \(\lambda\) defines a dimensionless synchronization strength. Thus, in-phase synchronized beating is stable with respect to perturbations. Dots mark the completion of a full beat cycle of the left flagellum. (Bottom) To mimic experiments where external forces constrain cell motion, we simulated the idealized case of a cell that cannot translate, while cell-body yawing is constricted by an elastic restoring torque \(Q_y = -k\alpha\) that acts at the cell body center (Top). Again, the two flagella synchronize (Middle) with a synchronization strength \(\lambda\) that can become even larger than in the case of a free swimming as shown here for \(k = 2 \times 10^7 \text{pN} \mu\text{m}\), which is close to the rotational stiffness for which the synchronization strength \(\lambda\) is maximal (Bottom). For very large clamping stiffness \(k\), the cell body cannot move and the synchronization strength \(\lambda\) attenuates to a basal value \(\lambda = 0.03\), which arises solely from direct hydrodynamic interactions between the two flagella (arrow). Parameters: \(2\pi/\omega_0 = 30\text{ ms}\).
hydrodynamic model. Their dependence on the flagellar phase is shown in Fig. 5 (Left). The physical significance of Eqs. 2–4 can be explained as follows. Eq. 2 implies that during the effective stroke of the left flagellum ($\rho \sim 0$), a counter-clockwise rotation of the whole cell slows down the flagellar beat, whereas a clockwise rotation speeds it up (Fig. 5B, $\mu \geq 0$). Eq. 3 implies the converse for the right flagellum. During the recovery stroke ($\rho \sim 180^\circ$), the effect is opposite and a counter-clockwise rotation of the cell would speed up the beat of the left flagellum ($\mu < 0$). Eq. 4 states that flagellar beating causes the cell body to yaw: If the right flagellum were absent, the model cell would rotate clockwise during the effective stroke of the left flagellum (Fig. 5A, $\nu > 0$), and counter-clockwise during its recovery stroke ($\nu < 0$). This swimming behavior is observed for unflagellar mutants (21). For synchronized beating of the two flagella, the right-hand side of Eq. 4 cancels to zero and the model cell swims straight. For asynchronous flagellar beating with a finite phase difference $\delta = \varphi_l - \varphi_r$, the phase dependence of the coupling function $\upsilon(\varphi)$ results in an imbalance of the torques generated by the left and right flagellum, respectively, which is balanced by a rotation of the whole cell.

We study the dynamical system given by Eqs. 2–4 after a small perturbation of the synchronized state at $t = 0$ with initial flagellar phase difference $0 < \delta(0) \ll 1$. For simplicity, we assume equal intrinsic beat frequencies, $\omega_l = \omega_r = \omega_0$. The synchronization strength $\lambda$ is given by $\lambda = \int_0^\pi dt \delta(\varphi)$ in the limit of a small elastic constraint, we find (Supporting Information)

$$\lambda = -2\int_0^\varphi \frac{\rho(\varphi)\upsilon'(\varphi)}{\rho(\varphi, \varphi) - \rho(\varphi, \varphi)} d\varphi$$

where a prime denotes differentiation with respect to $\varphi$. Using the coupling functions $\mu, \nu$, and $\rho$ computed above, we obtain $\lambda > 0$, which implies stable in-phase synchronization (Fig. 4). In the case of a stiff elastic constraint, we obtain a different result for $\lambda$:

$$\lambda = -2\int_0^\varphi \frac{\mu(\varphi)\upsilon''(\varphi)}{k/\omega_0} d\varphi$$

where $k > 0$, which implies stable in-phase synchronization (Eq. 4).

Synchronization in the absence of an elastic restoring force as characterized by Eq. 5, and synchronization involving a strong elastic coupling as characterized by Eq. 6 shows interesting differences, which relate to the fact that in the first case the flagellar phase dynamics depends only on the yawing rate $\dot{\varphi}$, but not on $\varphi$ itself. The difference between these two synchronization mechanisms is best illustrated in a special case, in which both the ratio $\sigma = \mu/\nu$ and $\rho$ are constant. A constant $\sigma$ corresponds to an active flagellar driving force that does not depend on the flagellar phase, whereas for constant $\rho$ the angular friction for yawing would not depend on the flagellar configuration. In the limit of a stiff elastic constraint, $k \gg \omega_0$, we readily find $\lambda = -\sigma_0 k \upsilon''/k = \sigma_0 k \upsilon''/k_0 > 0$, which indicates stable in-phase synchronization. In the limit of a weak elastic constraint, $k \ll \omega_0$, however, the integral on the right-hand side of Eq. 6 cancels to zero, which implies that synchronization does not occur. Hence, synchronization in the absence of an elastic restoring force requires that either $\mu/\nu$ or $\rho$ depend on the flagellar phase.

For our realistic Chlamydomonas model, $\mu$ and $\nu$ differ (Fig. 5A), and also $\rho$ is not constant (Fig. S3). This allows for rapid synchronization also in the absence of elastic forces. Previous work on synchronization in minimal models showed that elastic restoring forces can facilitate synchronization (11, 30). Here, we have shown that elastic forces can increase the synchronization strength (Fig. 4), but they are not required for flagellar synchronization in swimming Chlamydomonas cells, even if hydrodynamic interactions are neglected.

Our discussion of flagellar synchronization can be extended to the case, where the intrinsic beat frequencies of the two flagella do not match. If the frequency mismatch $|\omega_l - \omega_r|$ is small compared to the inverse time scale of synchronization, $1/T$, a general result implies that the two flagellar oscillators will still synchronize (7). For a frequency mismatch that is too large, the two flagella display phase drift with a phase difference that increases monotonously (18).

**Experiments Show Coupling of Beating and Yawing.** We reconstructed the coupling functions $\mu(\varphi)$ and $\upsilon(\varphi)$ between beating and yawing from experimental data using the theoretical framework developed in the previous section. In brief, (i) we extracted the instantaneous yawing rate $\dot{\varphi}$ and flagellar phase speeds $\varphi_l$ and $\varphi_R$ from high-speed videos of swimming Chlamydomonas cells, (ii) we represented the coupling functions by a truncated Fourier series, and (iii) we obtained the unknown Fourier coefficients by linear regression using Eqs. 2–4. The high temporal resolution of our imaging enabled us to accurately determine phase speeds as time derivatives of flagellar phase angle data. Fig. 5B displays averaged coupling functions obtained by fitting for a typical Chlamydomonas cell, fits for five more cells are shown in Figs. S6 and S7. We find a significant coupling between flagellar phase speeds and yawing rates, which are in good qualitative agreement with the theoretical predictions.

For the experimental conditions used, we commonly observed cells that displayed a large frequency mismatch between the two flagella. In the cells selected for analysis, this frequency mismatch exceeded 30%. This large frequency mismatch caused flagellar phase drift, which resulted in pronounced cell-body yawing and enabled us to accurately measure the coupling of yawing and flagellar beating. Experiments were done using either white-light illumination, which gave maximal image quality, or red-light illumination, which reduces a possible phototactic stimulation of the cells.

The observed modulation of flagellar phase speed according to the rate of yawing is consistent with a force–velocity dependence of flagellar beating, for which the speed of the beat decreases if the hydrodynamic load increases. We propose that a similar load characteristic of the flagellar beat holds also in cases of small frequency mismatch, where it allows for flagellar synchronization.
Conclusion and Outlook

We have presented a theory on the hydrodynamic coupling underlying flagellar synchronization in swimming *Chlamydomonas* cells. We have shown that direct hydrodynamic interactions between the two flagella as considered in refs. 9–11 give only a minor contribution to the computed synchronization strength and are unlikely to account for the rapid synchronization observed in experiments (16–19). In contrast, rotational motion of the swimmer caused by asynchronous beating imparts different hydrodynamic friction forces on the two flagella, which rapidly brings them back in tune: *Chlamydomonas* rocks to get into synchrony. Using high-speed tracking experiments, we could confirm the two-way coupling between flagellar beating and cell-body yawing predicted by our theory. The striking reproducibility of our fits for the corresponding coupling functions and their favorable comparison to our theory is highly suggestive of a regulation of flagellar phase speed by hydrodynamic friction forces that depend on rotational motion. Thus, coupling of flagellar beating and cell-body yawing provides a strong candidate for the mechanism that underlies flagellar synchronization of swimming *Chlamydomonas* cells. A similar mechanism may account for synchronization in isolated flagellar pairs (31) (Fig. S8).

To explain a previously observed synchronization for cells held in a micropipette (17–19), we propose a finite clamping compliance that still allows for residual cell-body yawing with an amplitude of a few degrees, which is sufficient for rapid synchronization. Alternatively, a compliant basal anchorage of the flagellar apparatus, which rapidly brings them back in tune, may account for the rapid synchronization observed in experiments (16–19). In contrast, rotational motion of the swimmer caused by asynchronous beating imparts different hydrodynamic friction forces on the two flagella, which rapidly brings them back in tune: *Chlamydomonas* rocks to get into synchrony. Using high-speed tracking experiments, we could confirm the two-way coupling between flagellar beating and cell-body yawing predicted by our theory. The striking reproducibility of our fits for the corresponding coupling functions and their favorable comparison to our theory is highly suggestive of a regulation of flagellar phase speed by hydrodynamic friction forces that depend on rotational motion. Thus, coupling of flagellar beating and cell-body yawing provides a strong candidate for the mechanism that underlies flagellar synchronization of swimming *Chlamydomonas* cells. A similar mechanism may account for synchronization in isolated flagellar pairs (31) (Fig. S8).

Materials and Methods

Hydrodynamic Computation of Swimming *Chlamydomonas*. We represent a *Chlamydomonas* cell by an ensemble of 300 spheres of radius $a = 0.25 \mu m$ (Fig. 2A) and use a freely available hydrodynamic library based on a Cartesian multipole expansion technique (27) to compute the grand hydrodynamic friction moment $\mathbf{M}$ for this ensemble of spheres. We assume a rigid cell body, and hence that the spheres constituting the cell body move as a rigid unit, which results in $n = 2, 14 = 1$ independently moving objects. The matrix $G$ has dimensions $6n \times 6n$ and relates the components of the translational and rotational velocities, $\mathbf{v}$ and $\mathbf{\Omega}$, of each of the $n$ objects to the hydrodynamic friction forces, $\mathbf{F}$, and $\mathbf{T}$, exerted by the $j$-th object on the fluid, $(F_{xj}, F_{yj}, F_{zj}, T_{xj}, T_{yj}, T_{zj}) = G_{ij} \{ q_{0j}, q_{1j}, \ldots, q_{6j} \} = G_{ij} \{ \mathbf{v}_{0j}, \mathbf{v}_{1j}, \mathbf{\Omega}_{0j}, \mathbf{\Omega}_{1j} \}$. Fig. 2C and D shows a submatrix of $G$ that relates force and velocity components parallel to the long axis of the cell body. The reduced friction matrix $\Gamma$ for a set of $m$ effective degrees of freedom $q$ is computed from $G$ as $\Gamma = L^T GL$ with $n \times m$ transformation matrix $L = [\mathbf{v}_{0j}, \mathbf{v}_{1j}, \mathbf{\Omega}_{0j}, \mathbf{\Omega}_{1j}, \ldots, \mathbf{\Omega}_{mj}]$. Initial tests confirmed that the friction matrix of only the cell body gave practically the same result as the analytic solution for the enveloping spheroid; similarly, the computed friction matrix of only a single flagellum matched the prediction of resistive force theory (26).

Imaging *Chlamydomonas* Swimming in a Shallow Observation Chamber. For cell culture, C. reinhardtii cells (CC-125 wild-type mt− 137C, R. P. Levine via N. W. Gilliam, 1968) were grown in 300 ml TAP+P buffer (32) (with 4x phosphate) at 24 °C for 2 d under conditions of constant illumination (two 75-W fluorescent bulbs) and constant air bubbling to a final density of 10^6 cells/mL. For high-speed video microscopy, an assay chamber was made of precleaned glass and sealed using Vitapur, a 1:1 mixture of lanolin, paraffin, and petroleum jelly, heated to 70 °C. The surface of that chamber was blocked using casein solution of casein micropipettes. We imagine a rigid cell body, and hence that the spheres constituting the cell body move as a rigid unit, which results in $n = 2, 14 = 1$ independently moving objects. The matrix $G$ has dimensions $6n \times 6n$ and relates the components of the translational and rotational velocities, $\mathbf{v}$ and $\mathbf{\Omega}$, of each of the $n$ objects to the hydrodynamic friction forces, $\mathbf{F}$, and $\mathbf{T}$, exerted by the $j$-th object on the fluid, $(F_{xj}, F_{yj}, F_{zj}, T_{xj}, T_{yj}, T_{zj}) = G_{ij} \{ q_{0j}, q_{1j}, \ldots, q_{6j} \} = G_{ij} \{ \mathbf{v}_{0j}, \mathbf{v}_{1j}, \mathbf{\Omega}_{0j}, \mathbf{\Omega}_{1j} \}$. Fig. 2C and D shows a submatrix of $G$ that relates force and velocity components parallel to the long axis of the cell body. The reduced friction matrix $\Gamma$ for a set of $m$ effective degrees of freedom $q$ is computed from $G$ as $\Gamma = L^T GL$ with $n \times m$ transformation matrix $L = [\mathbf{v}_{0j}, \mathbf{v}_{1j}, \mathbf{\Omega}_{0j}, \mathbf{\Omega}_{1j}, \ldots, \mathbf{\Omega}_{mj}]$. Initial tests confirmed that the friction matrix of only the cell body gave practically the same result as the analytic solution for the enveloping spheroid; similarly, the computed friction matrix of only a single flagellum matched the prediction of resistive force theory (26).

Supporting Information

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A. Image Analysis
High-speed movies were analyzed using custom-made Matlab software (MathWorks Inc); our image analysis pipeline is illustrated in Fig. S1. In a first step, estimates for position and orientation of the cell body in a movie frame were obtained by a cross-correlation analysis using rotated template images. In a second step, these position and orientation estimates were refined by tracking the bright phase halo surrounding the cell. The first and second area moments of the cell rim provide accurate estimates for the center of the cell body and its long orientation axis. While the tracking precision of the first step amounts to <500 nm for the position and a few degrees for the orientation, these values are reduced to <50 nm and <0.5° after the second step, respectively. Special care was taken to reduce any potential bias of the flagellar phase on the cell-body tracking; for example, the cell rim close to the flagellar bases was obtained by interpolation instead of direct tracking. The flagellar base is visible as a continuous, parabola-shaped curve that connects the proximal ends of the two flagella; tracking of this flagellar base was done by combinations of line scans and local fitting of a Gaussian line model (step 3). Flagella were tracked by advancing along their length using exploratory line-scans in a successive manner (step 4). Flagellar tracking can be refined by local fitting of a Gaussian line model. A movie consisting of 1,000 frames can be analyzed in an automated manner within 10 h on a standard personal computer. Movies from red-light illumination conditions were of lower quality and required manual correction of the automated tracking results for each frame.

B. Flagellar Shape Analysis
We employ a nonlinear dimension reduction technique to represent tracked flagellar shapes as points in a low-dimensional abstract shape space. In a first step, smoothed tracked flagellar shapes corresponding to one cycle of synchronized flagellar beating (shown in Fig. 1A) were used to define the basis of the shape space. Flagellar shapes can be conveniently represented with respect to the material frame of the cell using a tangent angle representation [1, 2]. In terms of this tangent angle $\theta(s)$, the $x(s)$ and $y(s)$ coordinates of the flagellar midline as functions of arclength $s$ along the flagellum can be expressed as

$$x(s) = x(0) + \int_0^s d\xi \cos(\alpha + \theta(\xi))$$

and

$$y(s) = y(0) + \int_0^s d\xi \sin(\alpha + \theta(\xi)).$$

Here, $\alpha$ is the orientation angle of the long axis of the cell body (Fig. 1A), which implies that $\theta(\xi)$ characterizes flagellar shapes with respect to a material frame of the cell body. By averaging the tangent angle profiles $\theta(s, t)$ over a full beat cycle, we define a time-averaged flagellar shape characterized by a tangent angle $\bar{\theta}(s)$. To characterize variations from this mean flagellar shape, we employed a kernel principal component analysis (PCA) [3]. The kernel used to compute the Gram matrix $D$ for the kernel PCA must account for the $2\pi$ periodicity of the tangent angle data and was taken as $D_{ij} = \int_0^2 ds \cos(\theta(s, t_i) - \theta(s, t_j))$. The first three shape eigenmodes account for 97% of the spectrum of $D$ and are shown in Fig. S2A. The relative contributions to the spectrum read 67% (first mode), 18% (second mode), and 12% (third mode). Whereas the first mode $\theta_1(s)$ (blue) describes nearly uniform bending of the flagellum, the second mode $\theta_2(s)$ (green) and the third mode $\theta_3(s)$ (red) together comprise the components of a traveling bending wave.

Next, any flagellar shape can be projected onto the shape space spanned by these three shape modes: Given a flagellar midline with coordinates $x(s)$ and $y(s)$, we seek the optimal approximating shape with coordinates $\hat{x}(s), \hat{y}(s)$ whose tangent angle $\hat{\theta}(s)$ is a linear combination of the fundamental shape modes:

$$\hat{\theta}(s) = \bar{\theta}(s) + \beta_1 \theta_1(s) + \beta_2 \theta_2(s) + \beta_3 \theta_3(s).$$  \[S2\]

The coefficients $\beta_1$, $\beta_2$, and $\beta_3$ are obtained by a non-linear fit that minimizes the squared Euclidean distance $\int_0^2 ds |(x(s) - \hat{x}(s))^2 + (y(s) - \hat{y}(s))^2|$. This procedure is robust and works even if flagellar shapes could only be tracked partway through the 272 tracked length $L$, shorter than the total flagellar length $L$. Note that for nonsmothered flagellar shapes the tangent angle representations can be noisy and are thus less suitable for fitting as compared to $x, y$ coordinates.

A time sequence of tracked flagellar shapes thus results in a point cloud in the shape space parameterized by the shape mode coefficients $\beta_1, \beta_2, \beta_3$. We fitted a closed curve to the torus-like point cloud (Fig. S2B, solid line). This closed curve represents a limit cycle of periodic flagellar beating. Each tracked flagellar shape can be assigned the “closest” point on this limit cycle (i.e., the point for which the corresponding flagellar shape has minimal Euclidean distance). By choosing a phase angle parameterization for the limit cycle, the phase angle of each flagellar shape is determined modulo $2\pi$. A time-series of flagellar shapes thus yields a time-series of the flagellar phase angle $\phi(t)$. The phase angle parameterization of the limit cycle had been chosen such that the flagellar phase angle $\phi$ and its time derivative are not correlated. Finally, the zero point $\phi = 0$ was chosen such that the corresponding flagellar shape was nearly straight and perpendicular to the long cell axis.

C. Computation of Hydrodynamic Friction Forces
For our hydrodynamic computations, we represented a Chlamydomonas cell by an ensemble of $N = 300$ equally sized spheres of radius $a = 0.25$ $\mu$m. The cell body was chosen spherical and is represented by 272 spheres that are arranged in a symmetric fashion to retain mirror symmetries. Each flagellum is represented by a chain of 14 spheres that are aligned along a flagellar midline with equidistant spacing. The shapes of the flagellar midlines depend on respective phase angles $\theta_L$ and $\theta_R$ for the left and right flagellum. These flagellar shapes were taken from experiment for one full period of synchronized beating and are shown in Fig. 1A. We measured the position and orientation estimates were re

\[P_0 = G \cdot \dot{q}_0. \]  \[S3\]

Here, $\dot{q}_0$ denotes a 6$\times$ vector that combines the translational and rotational velocity components of the n sphere clusters,
\[ \dot{q}_0 = (v_{1x}, v_{1y}, v_{1z}, \alpha_{1x}, \alpha_{1y}, \alpha_{1z}, \ldots, \alpha_{nz}), \]  
[S4]

whereas the 6n vector \( P_0 \) combines the components of the resultant hydrodynamic friction forces and torques,

\[ P_0 = (F_{1x}, F_{1y}, F_{1z}, T_{1x}, T_{1y}, T_{1z}, \ldots, T_{n_z}). \]  
[S5]

(Primed torques represent torques with respect to the center of the respective sphere cluster.) Fig. 2C in the main text shows a submatrix of the grand friction matrix, which was defined as \( G_{ij} = G_{i,i-4,j-4}, i, j = 1, \ldots, n \). In this figure, it was assumed that the long cell body axis is aligned with the y axis of the laboratory frame (i.e., \( \alpha = 0 \)), which implies that the submatrix relates motion in the direction of the long cell axis and the hydrodynamic force components projected on this axis.

For our hydrodynamic computations, the multipole expansion order was chosen as three. An estimate for the accuracy of our computation could be obtained by increasing the expansion order parameter, which changed the computed friction coefficients by less than 1%. Initial tests confirmed that the friction matrix of only the cell body gave practically the same result as the analytic solution for the enveloping spheroid (6); similarly, the computed friction matrix of only a single flagellum matched the prediction of resistive-force theory (7) assuming a flagellar radius equal to the sphere radius. Note that the precise value of the flagellar radius is expected to affect hydrodynamic friction coefficients only as a logarithmic correction (8).

Below, we consider an extension of the theoretical description given in the main text that additionally considers the possibility of an elastically anchored flagellar base, which allows for pivoting of the flagellar basal apparatus (Fig. S9). In this case, the flagellar midlines were rotated by an angle \( \psi \).

A set of 2,400 precomputed configurations was then used to construct a spline-based lookup table of the (reduced) hydrodynamic friction matrix as a function of the degrees of freedom \( q_{L}, \gamma_{L}, \) and \( \psi \). The interpolation error was confirmed to be on the order of 1% or less. This lookup table was then used for the numerical integration of the (stiff) equations of motion, Eqs. 1 and S17.

**D. Generalized Hydrodynamic Friction Forces**

We employ the framework of Lagrangian mechanics of dissipative systems (9) to define generalized hydrodynamic friction forces and derive an equation of motion for the effective degrees of freedom in our theoretical description of *Chlamydomonas* swimming and synchronization. The 6n degrees of freedom \( q_0 \) for the \( n \) sphere clusters used in our hydrodynamic computations are enslaved by the five effective degrees of freedom in our coarse-grained theory (Fig. 1). Below, one more degree of freedom, \( \psi \), is introduced to characterize pivoting of an elastically anchored flagellar basal apparatus. We thus have

\[ q_0 = q_0(\psi), \]  
[S6]

where we introduced the six-component vector \( q = (x, y, \alpha, \varphi_L, \varphi_R, \psi) \) that comprises the six effective degrees of freedom. The reduced 6x6 hydrodynamic friction matrix \( \Gamma \) for these six effective degrees of freedom can be computed from the grand hydrodynamic friction matrix \( G \)

\[ \Gamma = L^T \cdot G \cdot L \]  
[S7]

with a 6n x 6 transformation matrix \( L \) given by refs. 10 and 11:

\[ L_{ij} = \partial q_0/\partial \psi_j \]  
[S8]

The rate of hydrodynamic dissipation can now be equivalently written as a quadratic function of either \( \dot{q}_0 \) or \( \dot{q} \):

\[ R = \dot{q}^T \cdot G \cdot \dot{q}_0 \]  
[S9]

The generalized hydrodynamic friction coefficients \( \Gamma_{ij} \) are depicted in Fig. S3. In this context, generalized hydrodynamic friction forces can be defined as

\[ P_j = \Gamma_{ij} x_i + \Gamma_{ij} y_j + \Gamma_{ij} \alpha_i + \Gamma_{ij} \varphi_L + \Gamma_{ij} \varphi_R + \Gamma_{ij} \psi, \quad j = x, y, \alpha, L, R, \psi. \]  
[S10]

Interestingly, the generalized hydrodynamic friction force conjugated to one degree of freedom depends also on the rates of the change of the other degrees of freedom, which implies a coupling between the various degrees of freedom. This fact is illustrated by Fig. S4. Fig. S4A depicts the translational velocities of the flagellar spheres caused by pure yawing of the cell body with rate \( \dot{\alpha} \). This motion is characterized by a 6n vector of velocity components, \( \dot{q}_0(\alpha) = \{0, 0, 0, 0, 0, 0\} \). Similarly, the beating of the left flagellum induces hydrodynamic friction forces as shown in Fig. S4B. The resultant force (and torque) components are combined in the 6n vector \( P_0 = \{G \cdot L \cdot \{0, 0, 0, 0, 0, 0\}\} \). Fig. S4 indicates that the scalar product \( \dot{q}_0 \cdot P_0 = \alpha \dot{\alpha} \varphi_L \), does not vanish, which implies a nonzero friction coefficient \( \Gamma_{\alpha \alpha} \) and thus a coupling between cell-body yawing and flagellar beating.

In our theoretical description, the phase dynamics of the left flagellum, say, is governed by a balance of the generalized hydrodynamic friction force \( P_\alpha \) and an active driving force \( Q_\alpha \), similarly \( Q_\alpha = P_\alpha \) for the right flagellum. In the case of free swimming, force and torque balance imply \( P_\alpha = P_R = 0 \) and \( \alpha = 0 \). Together with an equation for \( P_\alpha \), these equation allow to self-consistently solve for the rate of change \( \dot{\psi} \) of the 6 degrees of freedom. If one degree of freedom were constrained, \( \dot{q}_i = 0 \), the corresponding force equation becomes void, since a constraining force \( Q_\alpha \) equal to \( P_\alpha \) then balances the generalized hydrodynamic friction force \( P_\alpha \) associated with this degree of freedom.

In general, the active driving forces \( Q_\alpha \) and \( Q_R \) will depend on the flagellar phase. This phase dependence is fully determined by the requirement that the flagellar phase speeds should be constant, \( \dot{\varphi}_L = \omega_0 \), in the case of synchronized flagellar beating with \( \delta = 0 \). Here, \( \omega_0 \) denotes the angular frequency of synchronized flagellar beating. Explicitly, we find

\[ Q_\alpha(\varphi_L) = \omega_0 \left[ \Gamma_{\alpha L}(\varphi_L, \varphi_R) + \Gamma_{\alpha R}(\varphi_L, \varphi_R) \right. \]
\[ \left. - 2 \Gamma_{\alpha L}^2(\varphi_L, \varphi_L) / \Gamma_{\alpha L}(\varphi_L, \varphi_L) \right]. \]  
[S11]

An analogous expression holds for \( Q_R(\varphi_R) \). Note that the generalized active driving forces are conjugate to an angle, and therefore have the physical unit piconewtons times micrometer. These phase-dependent active driving forces can be written as potential forces \( Q_j = -\partial U / \partial \varphi_j, j = L, R \), where the potential \( U \) reads

\[ U = - \int_{-\infty}^{\varphi_L} d\varphi_L Q_L(\varphi_L) - \int_{-\infty}^{\varphi_R} d\varphi_R Q_R(\varphi_R). \]  
[S12]

The potential \( U \) continuously decreases with time, indicating the depletion of an internal energy store and the dissipation of energy into the fluid during flagellar swimming. The rate of hydrody-
dynamic dissipation equals the rate at which potential energy is dissipated:

$$ R = -\dot{U} = Q_L \dot{\phi}_L + Q_R \dot{\phi}_R. \tag{S13} $$

E. Analytic Expression for the Flagellar Synchronization Strength

We present details on the derivation of Eqs. 5 and 6 for the synchronization strength \( \lambda \) in the case of the reduced equations of motion, Eqs. 2–4. We assume equal intrinsic beat frequencies, \( \omega_0 = \omega_R = \omega_L \), and a small initial phase difference, \( 0 < \delta(0) \ll 1 \).

To leading order in \( \delta \), we find relations that link the rotation rate \( \dot{\alpha} \) and the rate \( \dot{\delta} \) at which the phase difference changes,

$$ k \dot{\alpha} + \rho(\phi, \varphi) \dot{\alpha} = -d[\varphi(\phi) \delta]/dt \tag{S14} $$

$$ \dot{\delta} = -2 \mu(\phi) \dot{\alpha}. \tag{S15} $$

Here \( \varphi \approx \omega_0 \delta \) denotes the mean flagellar phase. The first equation describes how flagellar asynchrony causes a yawing motion of the cell body, and the second equation describes how this yawing motion then changes the flagellar phase difference. In the absence of any elastic constraint for yawing, \( k = 0 \), we can solve for \( \delta \):

$$ (\rho - 2 \mu \dot{\alpha}) \dot{\delta} = 2 \mu \omega_0 \delta. \tag{S16} $$

Now, Eq. 5 follows from Eq. S16 using \( \lambda = -\int_0^\delta dt \delta/\delta \) and a variable transformation \( \phi(t) = \omega_0 t + \mathcal{O}(\delta) \).

In the case of a very stiff elastic constraint with \( k \gg \omega_0 \delta \), we make use of the fact that variations of the phase difference \( \delta \) during one beat cycle will be small compared to its mean value \( \delta_0 = \langle \delta \rangle \). As a consequence, Eq. S14 can be approximated as \( k \dot{\alpha} \approx -\omega_0 \delta. \) Using this approximation and Eq. S15, Eq. 6 follows.

F. Comparison of Experiment and Theory

We can compare instantaneous swimming velocities predicted by our hydrodynamic computation with experimental measurements and find favorable agreement (Fig. 3 and Fig. S5). Note that wall effects present in our experiments, but not accounted for by our hydrodynamic computations, are expected to reduce translational velocities (but less so rotational velocities) (12). The hydrodynamic computations are based on a fixed flagellar beat pattern parameterized by a flagellar phase angle, which was obtained experimentally for one beat cycle with synchronized beating (Fig. L4). The good agreement between theoretical predictions and experimental measurements for the instantaneous swimming velocities further validate our reductionist description of the flagellar shape dynamics by just a single phase variable for each flagellum.

Next, we tested the applicability of the reduced equations of motion, Eqs. 2–4, in the experimental situation. For this aim, we reconstructed the coupling functions \( \mu_\phi(\phi), \nu_\phi(\phi) \) and \( \rho_\phi(\phi) \) from experimental time series data for \( \alpha, \dot{\phi}_L, \) and \( \dot{\phi}_R \). The coupling functions were represented by truncated Fourier series and the unknown Fourier coefficients determined by a linear regression of Eqs. 2, 3, or 4, respectively (Fig. S6). Repeating this fitting procedure for data from six different cells gave consistent results (Fig. S7). Moreover, the phase dependence of the fitted coupling functions agrees qualitatively with our theoretical predictions. Note that our simple theory does not involve any adjustable parameters.

G. An Elastically Anchored Flagellar Basal Apparatus

In the main text, we had assumed for simplicity that the flagellar base is rigidly anchored to the cell body. Whereas the proximal segments of the two flagella are tightly mechanically coupled with each other by so-called striated fibers to form the flagellar basal apparatus, the flagellar basal apparatus itself is only connected to an array of 16 long microtubules spanning the cell (13). We now consider the possibility that this anchorage allows for some pivoting of the flagellar basal apparatus as a whole by an angle \( \psi \) (Fig. S9A). In addition to the 5 degrees of freedom of Chlamydomonas beating and swimming considered in the main text (Fig. 1), we now include this pivot angle \( \psi \) as a 6th degree of freedom.

The rate of hydrodynamic dissipation is now given by \( R = \lambda \dot{\alpha} + \dot{\psi}_L + \omega_0 R\dot{\phi}_R + \dot{\phi}_L + \dot{\phi}_R + \psi \dot{P}_Y \), with \( \dot{P}_Y \) being the generalized hydrodynamic friction force conjugate to the pivot angle \( \psi \). Assuming Hookean behavior for the elastic basal anchorage with rotational pivoting stiffness \( \kappa \), we readily arrive at an equation of motion that reads in the case of free swimming:

$$ \begin{pmatrix} \dot{x} \\ \dot{y} \\ \dot{\alpha} \\ \dot{\psi}_L \\ \dot{\psi}_R \end{pmatrix} = \Gamma^{-1} \begin{pmatrix} 0 \\ 0 \\ 0 \\ Q_L \\ Q_R \end{pmatrix} - \kappa \dot{\psi}. \tag{S17} $$

Fig. S9B shows flagellar synchronization for a free-swimming cell with elastically anchored flagellar base: Although some basal pivoting occurs as a result of flagellar asynchrony, the swimming and synchronization behavior is very similar to the case of a rigidly anchored flagellar base, as shown in Fig. 4A. For a cell that can neither translate nor yaw, however, the situation is different (Fig. S9C). We find strong flagellar synchronization provided the elastic stiffness \( \kappa \) is not too large. Flagellar synchronization by basal pivoting is thus effective also for a fully clamped cell. In contrast, for a rigidly anchored flagellar base, the synchronization strength \( \lambda \) would be relatively weak in this case, being due only to direct hydrodynamic interactions between the two flagella.

Flagellar synchronization by basal pivoting is conceptually very similar to synchronization by cell-body yawing as discussed in the main text. In the case of a fully clamped cell, we can approximate the synchronization dynamics by virtually the same generic equation of motion as Eqs. 2–4, when we substitute \( \psi \) for \( \alpha \):

$$ \dot{\psi}_L = \omega_0 - \pi(\phi_L) \psi, \tag{S18} $$

$$ \dot{\psi}_R = \omega_0 + \pi(\phi_R) \psi. \tag{S19} $$

$$ \kappa \psi + \pi(\phi_L) \psi = -\tau(\phi_L) \psi_L + \tau(\phi_R) \psi_R. \tag{S20} $$

Here, the coupling functions \( \pi, \tau, \) and \( \pi \) play a similar role as the previously defined \( \mu, \nu, \) and \( \rho \) for Eqs. 2–4 and show a qualitatively similar dependence on the flagellar phase (Fig. S10). To derive Eqs. S18–S20, we neglected direct hydrodynamic interactions between the two flagella and approximated the active driving forces by \( Q_L(\phi) = \omega_0 L L(\phi, \phi_L) \psi_L \) and \( Q_R(\phi) = \omega_0 R R(\phi, \phi_R) \psi_R \). The coupling functions are defined as \( \pi(\phi, \psi) = -\Gamma(\phi, \psi)/\Gamma L(\phi, \phi_L) \psi_L \) and \( \tau(\phi, \psi) = -\Gamma(\phi, \psi)/\Gamma R(\phi, \phi_R) \psi_R \) (Fig. S3). Eq. S18 states that pivoting of the flagellar basal apparatus with \( \psi > 0 \) slows down the effective stroke of the left flagellum (and speeds up the right flagellum). For synchronized flagellar beating, there will be no pivoting of the flagellar base. For asynchronous beating, however, the flagellar base will be rotated out of its symmetric rest position by an angle \( \psi \) if the stiffness \( \kappa \) is not too large. Any pivoting motion of the flagellar base during the beat cycle changes the hydrodynamic friction forces that oppose the flagellar beat, which in turn can either slow down or speed up the respective flagellar beat cycles, and thus restore flagellar synchrony.

To gain further analytical insight, we studied the response of the dynamical system in Eqs. S18–S20 after a small perturbation \( 0 < \delta(0) \ll 1 \). To leading order in \( \delta = \phi_L - \phi_R \), we find (with \( \varphi \approx \omega_0 t \))

$$ \kappa \psi + \pi(\phi, \psi) \psi = -d[\varphi(\phi) \delta]/dt, \tag{S21} $$

$$ \dot{\delta} = -2 \tau(\psi) \psi. \tag{S22} $$

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In the biologically relevant case of a relatively stiff basal anchorage of the flagellar basal apparatus with $K \gg \rho \omega_0$, we find for the synchronization strength a result analogous to Eq. 6:

$$\lambda = -\frac{2\pi}{\int_0^{2\pi} d\phi \frac{P(\phi)\Phi(\phi)}{K/\omega_0}}.$$  [S23]
Fig. S1. Image analysis pipeline used to automatically track planar cell position and orientation as well as flagellar shapes in high-speed movies of swimming *Chlamydomonas* cells. (0) A typical movie frame. (1) Rotated template images used for a cross-correlation analysis to estimate cell position and orientation in a movie frame. (2) The cell body outline was tracked by detecting intensity maxima (green) of line scans along rays (shown in blue), which emanate from the putative cell-body center. From the cell-body outline, we obtain refined estimates for cell position and orientation. (2) The position of the flagellar base was then determined using a fan of line scans (along the blue lines), followed by a line scan (green) in a direction perpendicular to the maximal intensity direction (red). (4) Finally, flagellar shapes were tracked in a successive manner using combinations of line scans similar to those in step 3. (5) The final result of our tracking software provides for each frame: cell body position (red dot) and orientation (green arrow), cell body rim (green), as well as center lines of the two flagella (blue).
Fig. S2. We represent a single flagellar shape by \( n = 3 \) shape coefficients as a point in an abstract shape space that is spanned by three principal shape modes. (A) The principal shape modes were determined by employing a kernel PCA to the tangent angle representation \( \theta(s) \) of smoothed flagellar shapes that were tracked from the left flagellum of cell no. 2 during one beat cycle of synchronized flagellar beating. From the PCA, we obtained three dominant shape modes with respective tangent angle representations \( \theta_1(s) \), \( \theta_2(s) \), and \( \theta_3(s) \) as shown. Together, these principal shape modes account for 97% of the variance of this tangent angle dataset. For sake of illustration, exemplary flagellar shapes corresponding to the superposition of the mean flagellar shape and just one shape mode with tangent angle \( \theta_i(s) + \beta_i \theta_i(s) \), \( i = 1, 2, 3 \) are shown to the right (\(-5 \leq \beta_i \leq 5\)). (B) Each tracked flagellar shape from one flagellum can be represented by a single point in an abstract shape space that is spanned by the three principal shape modes. More specifically, the coordinates \((\beta_1, \beta_2, \beta_3)\) of this point are obtained by approximating the tracked flagellar shape by a superposition of a previously computed mean flagellar shape and the three principal shape modes (Eq. S2). The set of flagellar shapes from an entire experimental movie thus corresponds to a point cloud. This point cloud scatters around a closed curve (solid line), which reflects the periodic nature of the flagellar beat. This closed curve has been obtained by a simple fit to the point cloud of flagellar shapes and can be considered as a limit cycle of flagellar beating. Deviations from this limit cycle measure the variability of the flagellar beat. We can use this representation to define a distinct flagellar phase angle \( \varphi \) (modulo \( 2\pi \)) for each tracked flagellar shape as indicated by the color code by mapping each flagellar shape onto the limit cycle. A time series of flagellar shapes thus yields a time series of the flagellar phase angle \( \varphi(t) \). As an illustration of this assignment, superpositions of flagellar shapes are shown to the right, each of which corresponds to flagellar shapes that were assigned the same flagellar phase modulo \( 2\pi \). (C) Two-dimensional projections corresponding to the three-dimensional shape space representation in B.
is set to zero; different values of $\phi$ and $\Gamma_\alpha$ are conjugate to $\Gamma_0$ and $\Gamma_\psi$, respectively, as $\Gamma_\alpha$ is a periodic function of the two phase angles $\phi$ and $\Gamma_\psi$. Several features are noteworthy. The coefficients $\phi$ and $\Gamma_\alpha$ for the right $\phi$ are more pronounced way than the translational friction coefficients $\rho_\alpha$. By Onsager symmetry, $\Gamma_{ij}=\Gamma_{ji}$, and is represented as a color plot with axes as indicated. Here, $\alpha$ is set to zero; different values of $\alpha$ would correspond to a simple rotation of the matrix shown. Analogous statements hold for the coefficients $\Gamma_{ij}$. A counter-clockwise rotation of the cell, $\alpha>0$, will increase the friction force $P_\alpha$ during the effective stroke of the left flagellum $(\Gamma_\alpha>0)$ but decrease the corresponding respective friction force $P_\theta$ for the right flagellum during its effective stroke $(\Gamma_\theta<0)$. Mirror symmetry of the swimmer amounts to invariance of the friction matrix under the substitution $(x,y,\phi,\Gamma_\phi) \rightarrow (-x,-y,-\phi,\Gamma_\phi)$, which implies a number of symmetry relations, for example, $P_\alpha=\Gamma_{xx}$ must be symmetric in $\phi_\alpha$ and $\phi_\psi$. Finally, this rotational friction coefficient $P_\alpha=\Gamma_{xx}$ depends on the flagellar phases in a more pronounced way than the translational friction coefficients $\Gamma_{xx}$ and $\Gamma_{x\phi}$. This is in line with the general fact that rotational friction coefficients depend more strongly (as $\sim J^2$) on the effective linear dimension $l$ of an object than translational friction coefficients ($\sim l$). The coefficients $\Gamma_{xy}$ and $\Gamma_{yx}$ associated with yawing of the whole cell and pivoting of the flagellar apparatus, respectively, show a similar dependence on the flagellar phases.

**Fig. S3.** Generalized hydrodynamic friction matrix $\Gamma_{ij}$ associated with the effective degrees of freedom $x, y, \phi, \Gamma_\alpha$, and $\psi$. This generalized friction matrix determines the generalized hydrodynamic friction forces $P_i$ conjugate to the degrees of freedom $q=(x,y,\phi,\Gamma_\alpha,\psi)$ as $P_i=\Gamma_{ij}q_j$, and is computed as a projection of the grand hydrodynamic friction matrix (Eq. 57). Each friction coefficient $\Gamma_{ij}$ is a periodic function of the two phase angles $\phi$ and $\Gamma_\psi$. $\Gamma_{ij}=\Gamma_{ji}(\phi_\alpha,\phi_\psi)$ and is represented as a color plot with axes as indicated. Here, $\alpha$ is set to zero; different values of $\alpha$ would correspond to a simple rotation of the matrix shown. By Onsager symmetry, $\Gamma_{ij}=\Gamma_{ji}$. Several features are noteworthy. The coefficient $\Gamma_{ij}$ characterizes hydrodynamic interactions between the two flagella and is found to be small compared to, for example, $\Gamma_{ij}$. The other coefficients $\Gamma_{ij}=\Gamma_{ji}$ which set the friction force $P_i$ conjugate to $\phi_j$, depend strongly on $\phi_j$, but almost not on $\phi$. This is yet another manifestation of the fact that direct hydrodynamic interactions between the two flagella are comparably weak.

**Fig. S4.** Coupling of cell-body yawing and flagellar beating. (Left) Translational velocities of the flagellar spheres used in our hydrodynamic computation associated with a pure yawing motion of the cell body with rate $\alpha$. (Right) Hydrodynamic friction forces exerted by the flagellar spheres (as well as by the cell body), if the left flagellum advances along its beat cycle with rate $\phi_\alpha$. The generalized hydrodynamic friction coefficient $\Gamma_{ij}$ that couples cell-body yawing and beating of the left flagellum can be computed as a scalar product between the velocity profile resulting from yawing and the force profile resulting from flagellar beating and is found to be non-zero. Parameters: $\phi_\alpha=\omega_0$, $\alpha=0.2\omega_0$, and $2\pi/\omega_0=30$ ms.
Fig. 55. (A) Instantaneous swimming velocity in the direction perpendicular to the long cell axis as a function of the flagellar phase angles \( \phi_L \) and \( \phi_R \). For synchronized flagellar beating (dashed line), this velocity vanishes in our theory for symmetry reasons (green). If the two flagella are out of synchrony, however, significant sideward motion of the cell is observed, both in theory and experiment. Note that wall effects present in the experiments, but not considered in the computations, reduce translational velocities. (B) Instantaneous swimming velocity in the direction of the long cell axis, again as a function of the flagellar phase angles.

Fig. 56. The reduced equations of motion, Eqs. 2-4, were fitted to experimental time-series data for the yawing rate \( \alpha \) of the cell, as well as the flagellar phase speeds \( \phi_L \) and \( \phi_R \). This provided experimental estimates for the phase-dependent coupling functions \( \mu, \nu, \) and \( \rho \). Specifically, we represented each coupling function as a truncated Fourier series and determined the unknown coefficients by a linear regression using Eqs. 2-4. (A) Linear regression of Eq. 2. Shown to the left in black is the instantaneous flagellar phase speed of the left flagellum \( \phi_L \) (smoothed with a span of 15 ms). Shown in red is a reconstructed phase speed \( \omega_0 - \mu(\phi_L)\dot{\alpha} \) that depends on the instantaneous cell-body yawing rate \( \alpha \), as well as the intrinsic flagellar frequency \( \omega_0 \) and phase-dependent coupling function \( \mu \) for the left flagellum, which were obtained by the fit. The coefficient of determination was \( R^2 = 30\% \). The estimate for \( \mu_L \) obtained from this fit is shown to the right (blue), together with a theoretical prediction (black) (see also Fig. 5A). (B) Linear regression of Eq. 4. Shown to the left is the measured instantaneous yawing rate \( \alpha \) of the cell body (black) and a yawing rate reconstructed from the flagellar phase dynamics, \( -\nu(\phi_L)\dot{\phi}_L + \rho(\phi_L, \phi_R)/(\rho_0) \) (red). The coefficient of determination was \( R^2 = 93\% \). From this fit, we obtain an experimental estimate for the coupling functions \( \nu(\phi) \) and \( \rho(\phi_L, \phi_R) \) (blue); theoretical predictions are shown in black.
Fig. S7. Experimental fits for the coupling functions $\mu$, $\nu$, and $\rho$ introduced in Eqs. 2–4 (blue curves, shaded regions indicate mean ± SE) and theoretical predictions (black). The coupling functions $\mu$, $\nu$, and $\rho$ relate flagellar beating and cell-body yawing. Fitting results are shown for six different cells illuminated by either white or red light as indicated. For each cell, we employed $n$ fits using $n$ nonoverlapping time series of duration 0.4–0.5 s with $n$ as indicated. The blue curves represent the average of the fitted coupling functions for the $n$ fits; the averaged coefficient of determination $R^2$ is stated.

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Fig. S8. Theory of flagellar synchronization for an isolated flagellar pair. Inspired by experiments by Hyams and Borisy (1) reporting synchronization in isolated flagellar pairs, we computed the swimming and synchronization behavior of a flagellar pair with cell body removed. For the computations, we used flagellar shapes and flagellar driving forces $Q_j(\phi), j=L,R$, determined from an intact cell (Fig. 1) (A) For a free-swimming flagellar pair, we observe a characteristic yawing motion of the flagellar pair characterized by $\alpha(t)$, if the two flagella are initially out of synchrony. The flagellar phase difference $\delta$ is found to decrease with time (solid line), approximately following an exponential decay (dotted line). This implies that the in-phase synchronized state is stable with respect to perturbations. Each completion of a full beat cycle of the left flagellum is marked by a dot. (B) To mimic experiments in which external forces constrain the motion of the flagellar pair, we simulated the idealized case of a pair that cannot translate, while yawing of the pair is constricted by an elastic restoring torque $Q_{\alpha} = -k\alpha$ that acts at the basal apparatus (red dot). As in the case of a free-swimming pair, the flagellar phase difference $\delta$ decays with time, indicating stable synchronization. In the case of a constrained cell, the synchronization strength $\lambda$ strongly depends on the clamping stiffness $k$. Parameters: $2\pi/\omega_0 = 30$ ms, $k = 10^4$ pN $\mu$m rad. To enhance numerical stability, we added a small constant $\kappa = 10$ pN $\mu$m ms to the flagellar friction coefficients, $\Gamma_j(\phi_L,\phi_R)$, $j=L,R$, which corresponds to internal dissipation (2).

Fig. S9. Theory of flagellar synchronization by basal pivoting. (A) We consider the possibility of an elastically anchored flagellar basal apparatus (red), which allows for pivoting of the basal apparatus (solid lines) by an angle $\psi$ from its symmetric reference configuration (dashed lines). (B) For a free-swimming cell, the equation of motion, Eq. S17, predicts both a yawing motion of the cell characterized by $\alpha(t)$ and a pivoting motion of the flagellar base characterized by $\psi(t)$, if the two flagella are initially out of synchrony. The flagellar phase difference $\delta$ is found to decrease with time (solid line), approximately following an exponential decay (dotted line). This implies that the in-phase synchronized state is stable with respect to perturbations. Each completion of a full beat cycle of the left flagellum is marked by a dot. The synchronization behavior in the case of an elastically anchored flagellar basal apparatus is nearly identical to the case of a stiff anchorage, as shown in the main text in Fig. 4. The lowest panel shows typical amplitudes of basal pivoting ($\delta\psi$, solid line) and cell-body yawing ($\delta\alpha$, dashed line) as a function of basal stiffness $\bar{k}$. Amplitudes were determined as half the range of variation during one beat cycle for an initial phase difference of $\delta(0) = \pi/2$. (C) For a clamped cell that can neither translate nor rotate, the flagellar apparatus can still pivot and will do so if the two flagella are initially out of phase. As in the case of a free-swimming cell, the flagellar phase difference $\delta$ decays with time, indicating stable synchronization. In the case of a clamped cell, the synchronization strength $\lambda$ strongly depends on the stiffness $\bar{k}$ of the elastic anchorage of the basal flagellar apparatus, which sets the amplitude of basal pivoting. Parameters: $2\pi/\omega_0 = 30$ ms, $\bar{k} = 10^4$ pN $\mu$m/rad.
Fig. S10. Theoretical coupling functions for the case of a pivoting flagellar base. (A) A pivoting motion of the flagellar base changes the hydrodynamic friction force associated with flagellar beating and thereby speeds up or slows down the flagellar beat cycle in our theory. This effect is quantified by a coupling function $\mu$ (Eq. S18). (B) Hydrodynamic friction associated with pivoting of the flagellar base (and the attached flagella) is characterized by a friction coefficient $\rho$ (Eq. S20). This friction coefficient is maximal when the two flagella extend maximally from the cell body during their effective stroke. (C) The beat of the left flagellum causes pivoting of the flagellar base. This effect is quantified by a coupling function $\nu$ (Eq. S20).