Collective sinking promotes selective cell pairing in planktonic pennate diatoms

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Finding a partner in an inherently unsteady 3-dimensional system, such as the planktonic marine environment, is a difficult task for nonswimming organisms with poor control over their orientation. We experimentally investigate the process of cell pairing in pennate marine diatoms and present field evidence of its occurrence in the ocean. We describe the mechanism as a 3-step process in which pennate diatoms (i) vertically reorient while sinking from surface turbulent waters to a more stable environment (i.e., under the seasonal pycnocline), (ii) segregate from incompatible partners (e.g., dead or different sized cells), and (iii) pair with other partners as a result of the hydrodynamic instabilities generated by collective cell sinking. This is, eminently, a cell abundance-dependent process, therefore being more effective when population sinking is synchronized. We suggest that this selective process, enabling matching of size-compatible healthy partners, could be fundamental in understanding sexual reproduction in pennate diatoms.

diatoms | life cycle | collective sinking

Diatoms are a ubiquitous nonmotile phytoplankton group present in most water bodies that play a fundamental role in oceanic cycling of carbon and biogeochemical cycles (1, 2). They are distinguished by being encased inside 2 ornamented silica cell shells (frustules) that conform to their body (3). Diatoms are far by the most successful lineages of eukaryotic phytoplankton, not only in numbers of species but also in amount of biomass and in their contribution to global inorganic carbon fixation in the ocean (4) due to their highly efficient CO2 uptake mechanism (5). Indeed, it is estimated that this eukaryotic microalgal group is responsible for ~20% of the net primary production in the ocean and up to 50% of the organic carbon exported to the ocean interior (6, 7).

Diatom populations undergo periods of rapid growth in turbulent and nutrient-rich environments, often dominating phytoplankton assemblages in high latitudes and coastal areas (8, 9). These “blooms” are normally terminated by rapid mass sinking when environmental conditions become unfavorable, contributing greatly to the vertical fluxes in the ocean (10). Opportunistic dominance of phytoplankton assemblages by diatoms is mostly accomplished through a prolonged period of vegetative cell division (asexual phase) when the parental cell produces 2 daughter cells (11). This clonal reproduction via mitosis is generally characterized by a progressive reduction in the mean cell size of a population imposed by their rigid frustule. Therefore, after successive vegetative divisions, the size decreases below a certain threshold known as the first cardinal point (30 to 75% of initial size (3, 12)). To avoid mortality, the maximal size is typically restored by auxospore formation during obligated, but episodically occurring, sexual reproduction. This short sexual stage also has important benefits in terms of genetic recombination, elimination of deleterious mutations, and evolutionary adaptation (13, 14). Therefore, sporadic sexual mating events are a critical period determining the ecological dynamics of diatoms (11), and their failure during prolonged periods (i.e., a few years) can result in extinction of local populations (15).

Regarding the frustule morphology, 2 major morphological groups are prevalent: centric forms with disk-like bodies (radial symmetry) and elongated (bilaterally symmetrical) pennate diatoms (16, 17). Beyond their morphological differentiation, a major distinction between these groups is their pattern of sexual reproduction. Centric cells are characterized by oogamous reproduction, involving the formation of uniflagellate male gametes (sperms) that facilitate encounters with the nonmotile female gametes. Conversely, sexual reproduction in pennate diatoms depends on cell-to-cell interactions that involve valve-to-valve pairing. This implies that centric diatoms are capable of self-fertilization, while mating in most pennates promotes, per definition, cross-fertilization (18). An additional advantage is that pennates do not need to invest in many smallflagellated gametes, most of which will go to waste (4). However, while the search for a partner and alignment for sex may be well suited for benthic environments, it is more puzzling to conceive for organisms suspended in a 3-dimensional system, such as the pelagic environment, without a cell encounter-facilitating mechanism.

Sexual mating events in marine diatoms are relatively rare and elusive, occurring at intervals of >1 y during highly constrained periods (3). Therefore, evidence of pairing events in the natural environment during these episodes is scarce, and, as a consequence, little is known about the way this critical mechanism occurs in the water column. It is speculated that pairing in pennate diatoms involves some sort of buoyancy-associated movement since, in the absence of a process that transports cells relative to each other, randomly distributed cells cannot form aggregations, even if turbulent dispersion is spatially variable (19, 20).

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Based on previous theoretical analysis and experimental observations on high-aspect ratio particles (21), Botte et al. (22) proposed an original mechanism that would favor pennate diatom encounters in the marine environment: While sinking in calm hydrodynamic conditions, they should come closer to each other because of the specific hydrodynamic interactions that develop in such situations. In such case, the flow induced by the particles while sinking leads to a positive feedback between cell horizontal displacements and regions of high particle density (SI Appendix, Fig. S5), which, in turn, amplifies any minor inhomogeneity in their spatial distribution (23). Moreover, physical experiments on inert fibers (21) have shown that this clustering is accompanied by a preferential vertical alignment of the individual particles. However, a mechanistic demonstration of this process in pennate diatoms is lacking.

Results

To understand the mechanism of cell pairing, we first analyzed the patterns of orientation of living and dead cells while sinking in seawater. The reorientation patterns were tracked in independent experiments using both laser diffractometry and videomicroscopy. We studied the existence of active mechanisms in the control of cell orientation and buoyancy using the fusiform morphotype of Phaeodactylum tricornutum as a model system. Reorientation and sinking were analyzed at a low Reynolds number ($\sim 10^{-5}$) and in the dilute regime $n(l/2)^2 < 1$, with $n$ being the number of cells per unit volume and $l$ their characteristic length. The particle size distribution of $P. tricornutum$ from laser diffractometry measurements typically presents high variance at 2 differently sized bands: 1.89 to 3.11 μm ($r_2$) and 5.11 to 9.98 μm ($r_1$) (Fig. 1B). These size bands agree well with the apparent size of the transapical axis (2.3 ± 1 μm) and apical axis (10.6 ± 2.5 μm) of $P. tricornutum$ (i.e., high optical contrast area), while the actual axes span 3.0 ± 0.3 μm and 22.5 ± 2.8 μm, respectively, as measured using microscopy. We used the relative variation of the bands in the diffractometer signal (R) (Fig. 1C) to infer the orientation parameter ($\varepsilon$).

Fig. 1A shows the time evolution of cell orientation in our diffractometry experiments as depicted from $\varepsilon$ values. Starting from a random cell orientation ($\varepsilon = 0$) generated by intense stirring (stage 1), $P. tricornutum$ cells rapidly reorient horizontally ($\varepsilon \approx 0$) after stirring is ceased (stage 2). Cell reorientation during this stage is the result of alignment with the direction of remnant flow and lasts from 100 to $t_0=200$ s in living cells and for a shorter period in the case of dead cells ($t_0=150$ s). As the flow slows down even further due to viscous dissipation (stage 3), cells suddenly start to reorient parallel to gravity, with the major axis parallel to the incident beam ($\varepsilon > 0$, vertical orientation). This transition extends for nearly 450 s in living cells and 150 s in dead ones.

Fig. 1B shows the fluid conditions during these laser diffractometry experiments. Stage 1 is characterized by intense anomalies in the velocity field (6 U) caused by vigorous stirring. These variations from the mean vertical flow (SI Appendix, Fig. S1), together with the overall high levels of shear present during stirring, are responsible for the sustained random orientation ($\varepsilon = 0$) of cells observed in Fig. 1. Surprisingly, dead cells present a slight vertical alignment during agitation ($\varepsilon = 0.16$), pointing to an anomalous response to local shear. Fluid flow (and the corresponding mean shear, $\gamma$) exponentially decrease due to viscous forces in stage 2 (no stirring), and the anomalies in the velocity field smear out. For sufficiently low $\gamma$, cells begin to reorient vertically. The observed critical shear, $\gamma_c$, is compatible with a simple balance of torques argument where displacement of the cell center of mass hinders the archetypical Jeffery orbits expected in the dynamics of ellipsoids under fluid shear (24, 25). For an ellipsoid of aspect ratio, $AR$, and characteristic reorientation time, $\tau$, this balance is given by $\gamma_c = (4R^2 + 1)/2\gamma_c$. With $AR = 7.5 \pm 1.2$ and $\tau \approx 523 \pm 5$ s for living cells (SI Appendix, Fig. S2), $\gamma_c = 0.054 \pm 0.028$. As shown in Fig. 2, dead cells start orienting themselves at a higher critical shear value ($\gamma_c = 0.1 \pm 0.03$) than living cells, which is, again, compatible with their shorter reorientation times ($\tau \approx 279 \pm 1$). Videomicroscopy experiments corroborate cell reorientation with the apical axis parallel to the axis of gravity (SI Appendix, Fig. S2). In these experiments, vertical orientation not only occurs faster in dead cells (twice as fast) but is also more generalized (affects a larger percentage of the population). A similar response in both cell types indicates that reorientation is driven by a physical process rather than by cell behavior.

Pairing is tightly coupled to the orientation phenomenon, with both processes occurring consecutively during sinking (Fig. 3A). A principal component analysis of the size spectrum temporal variation in diffractometry experiments reveals that the first, and...
Critical shear conditions. (Upper) Anomalies in the velocity field ($\delta U$) at 2 different experimental times obtained using PIV during the orientation experiments shown in Fig. 1. (Lower) Temporal evolution of the mean shear ($\gamma$, dark blue) and adjustment of its exponential decay with time (magenta). The numbers correspond to the stages indicated in Fig. 1. Critical $\gamma$ values for the initiation of vertical cell orientation of living and dead cells are indicated in light and dark green.

Fig. 2. Critical shear conditions. (Upper) Anomalies in the velocity field ($\delta U$) at 2 different experimental times obtained using PIV during the orientation experiments shown in Fig. 1. (Lower) Temporal evolution of the mean shear ($\gamma$, dark blue) and adjustment of its exponential decay with time (magenta). The numbers correspond to the stages indicated in Fig. 1. Critical $\gamma$ values for the initiation of vertical cell orientation of living and dead cells are indicated in light and dark green.

As shown in Fig. 3C, our experimental results fit exceptionally well with the expected scaling with cell concentration. Video microscopy experiments are also coherent with this theoretical prediction showing that coagulation time depends not only on cell concentration but also, inversely, on sinking speed [with $\zeta$ in dead cells, which consistently present a less stable response to physical forcing and show significant faster sinking speeds (Fig. 3B), fitting on the same scaling law when rescaled by $V^2$]. Cell clustering, as revealed by the structure function, $g(r)$, increases with time ($SI$ Appendix, Fig. S5), indicating that cell contacts are not merely a result of random spatial arrangements but, instead, are generated by fluid-mediated collective interactions.

Field observations of cell orientation obtained using in situ laser diffractometry during a *Pseudo-nitzschia* bloom reveal a nonrandom cell orientation in the water column and an increase in cell pairing with enhanced vertical cell alignment. As shown in Fig. 4, *Pseudo-nitzschia* sp. cell abundances exceeding $10^6$ cells per liter were found at depths of 30 to 40 m, beneath the seasonal pycnocline. These pennate diatoms were characterized by an apical axis of $60 \pm 12 \mu$m and minor axis of $6 \pm 3 \mu$m. The depth variation of $\gamma$ (Fig. 4D) displays a random orientation in cells at shallower depths but progressive reorientation below 30 m to reach values consistent with unequivocal vertical alignment ($\gamma > 0$). At this depth, vertical shears did not exceed 0.007 s$^{-1}$. Both diffractometry measurements and visual microscopy counts revealed a higher proportion of paired cells (19%) at depths where increased vertical cell alignment was observed (Fig. 4D).

Discussion

Several studies have previously speculated that sinking of elongated cells at a low Reynolds number increases cell-to-cell contact probabilities, therefore favoring sexual reproduction in diatoms (13, 22, 26). However, experimental proof of the cell pairing mechanism and field evidence of its occurrence in the natural environment have been lacking. Here, we have experimentally demonstrated the existence of such a coagulation mechanism during sinking of pennate diatoms in the laboratory, and we have unambiguously provided its direct observation in the sea. Importantly, this pairing mechanism entails a preferential vertical orientation that favors cell-to-cell alignment along the apical axis, an obligate requirement for sexual exchange.

The conjecture of Botte et al. (22) is based on theoretical and experimental studies on dilute fiber suspensions settling at a low Reynolds number in unstratified fluids, showing concentration instabilities leading to particle clustering from their collective hydrodynamic interactions (21, 23, 27, 28). Nonetheless, most knowledge on the physics of coagulation emerges from experiments performed with inert particles of large sizes ($10^2$ to $10^4 \mu m$). We
Here, we propose that the combination of collective sinking and gravitational reorientation constitutes a robust mechanism for explaining pennate cell pairing in the sea. Crucially, such a mechanism is also very compatible with the life cycle and population dynamics of pennate diatoms. First, coagulation is a generic physical process solely driven by sinking, on which diatoms have a great control. Buoyancy regulation by osmotic variation of internal inorganic ion density (33, 34) is indeed a long-known mechanism by which diatoms physically control their interaction with the environment (35), influencing therefore their abundance and distribution in the water column. This ability helps notably regulating light and nutrient uptake (36–38). In fact, daily buoyancy-regulated migratory behavior has been observed in some diatoms that sink below the pycnocline to stock nutrients and then rise into the photic zone to osmosynthetize, using up the nutrient stock (35), while in the case of a sinking response upon depletion of ambient nutrients is observed in different marine diatom species (39, 40). This vertical migration pattern following diatom blooms is thought to be a survival strategy whereby cells accumulate in darker, colder, and nutrient-rich water and are then ready to reenter the surface mixing layer, seeding a genetically renewed population when conditions become favorable.

Secondly, coagulation by sinking is an inherently selective process favoring interactions between healthy conspecific cells of similar size, which is a requirement for successful sexual reproduction. Because of the peculiarities of mitotic division, in which each daughter cell inherits a parental shell, variance in cell size on diatom populations increases over time. However, as illustrated in Fig. 3A, cell pairing is a sequential process in which a vertical segregation induced by sinking speed variability precedes pairing between cells sinking at similar rates. This is due to the fact that the velocity of vertical cell segregation depends on the concentration of cells and on their sedimentation rates. For usual cell abundances during a bloom (e.g., 10⁶ cells per liter) and with the sinking speeds measured in our experiments, the segregation time between living and dead P. tricornutum is 12 orders of magnitude faster than the estimated coagulation time. Only in the case of unusually high cell abundances (e.g., >10⁷ cells per liter in the case of Pseudo-nitzschia) would the segregation and coagulation times be similar. Consequently, cells of the same size are more likely to interact, provided that their sinking speed is similar.

For cells of similar size, changes in sedimentation velocity rely on several internal and external factors. For instance, nutrient-depleted cells sink more rapidly than nutrient-replete ones (41, 42). Likewise, other health-related factors also affect buoyancy; for example, parasitized cells are known to sink faster, hindering propagation of infections (43). These variations in sinking velocity may indeed selectively increase encounter rates among healthy cells.

Other physical coagulation processes, including Brownian motion and turbulent shear, can also bring suspended particles together, but they are slow (over 10⁵-fold slower at typical bloom conditions), nonspecific (enhancing the encounter between particles of different shapes, sizes, and sinking speeds), and do not preserve cell alignment. Hence, they constitute an unlikely mechanism for pairing and, instead, are mostly relevant for larger scale aggregations during the termination of diatom blooms and the formation of marine snow (44–46). Moreover, long-range turbulent transport (and its associated coherent structures) might also increase local cell abundance via, for example, preferential concentration, indirectly shaping encounter rates (47).

Lastly, coagulation during cell sinking also entails a high degree of synchronicity. This may be critical for sexual reproduction because gametes have to selectively interact with each other. In pennate diatoms, the main factor initiating sexualization is cell size (48), but density-dependent mechanisms have been also observed (49). Several other cues have been regarded to trigger synchronous sexualization, including irradiance, temperature, ambient nutrient concentrations, and chemical signals (50, 51).

Pairing success in diatoms might be eventually determined by other complex biological factors and selection mechanisms. For instance, chain-forming species, for which contacts are expected to be more frequent than for individual cells, are known to
dramatically vary their sinking behavior (52). In addition, the existence of interlocking silica projections, spines, or tubes and the use of mucilage pads might all contribute to the ability of chains to retain proximity with compatible cells. Moreover, diatoms seem to have refined communication capabilities that can be used to vary the production of pheromones as well as other metabolites, such as allelopathic substances (53, 54). Therefore, although we have found pairing is greatly facilitated by collective cell-fluid interactions, it cannot be excluded that other active factors (Movie S2), such as chemical signaling, ultimately determine partner selection.

**Methods**

**Cell Culture.** _P. tricornutum_ cells were cultured in Erlenmeyer flasks containing 200 mL of Guillard's f/2-medium + silica (15 mg L−1) at 20 °C under a 16-h-light/8-h-dark cycle (OSRAM Fluora; 100 µmol−1·s−1 photosynthetically available radiation). Cultures were transferred once a week (dilution factor $x$200) to keep cells healthy and in the exponential growth phase.

**Fluidic System and Microscopy.** Primary molds of the fluidic channels were created by gluing a flat capillary (CM Scientific no. 5012-050; inner dimensions: 0.10 × 2.00 mm, length: 5 cm) onto a glass slide with optical ultraviolet glue (Norland Optical Adhesive 81). Polydimethylsiloxane (PDMS) was then poured on the mold and cured for 30 min at 90 °C to create straight channels with final dimensions of $0.340 × 2.4 × 50$ mm. Following the punching of holes at both ends of the channel, the piece of PDMS was irreversibly bonded to a glass slide using an air plasma etcher (CUTE; FemoScience). The channel was then held vertically on an optical table (Scienceequipment 89000Ach; Thorlabs) at the focus point between a dark-field light-emitting diode (LED) ring (580-55; Schott) and a continuously focussable microscope objective (100× for detection of each particle). The projection of each particle on the back of which a 4-megapixel (MP) complementary metal-oxide semiconductor (CMOS) microscope (UIM-3370PF-NIR-GL R2; IDS) was fixed. The top inlet was then connected to a gas-tight syringe (500 µL, model 1750; Hamilton) attached to a syringe pump (Fusion 200; Chemyx), while the other end of the channel was connected to a 50-L Falcon tube containing 10 to 20 mL of cell suspension. For all of the experiments, the cells contained in the Falcon tube were sucked into the PDMS channels at a flow rate of 50 µm−1·s−1 for 2 min using the syringe pump. This process oriented the cells perpendicular to the flow (i.e., perpendicular to gravity) and produced relatively uniform cell distributions. Soon after stopping the flow, the sinking dynamics were recorded at 2 frames per second (fps) for 1 h (living cells) or 30 min (dead cells, killed by adding 1% [vol/vol] of Lugol’s iodine solution) to extract the evolution of both the distribution of cell orientation and suspension structure (i.e., radial distribution function). To get sufficient statistics, this operation was repeated between 10 and 20 times depending on the specific experiment. Cell trajectories were digitized using a standard MATLAB particle-tracking algorithm (http://site.physics.georgetown.edu/matlab/).

Following tracking, cell orientation was obtained by analyzing the images further. This was done by cropping the images around the tracked features; thresholding and binarizing; and, finally, fitting the binary contours with ellipses. The radial distribution function is a unique inverse function (i.e., $c = 0$), solving numerically for it in the equation $R(\alpha, \beta) = R_{\text{meas.}}$. Once $R_{\text{meas.}}$ is known, it provides a functional route map for transforming size distributions into equivalent angular distributions; that is, it provides the unique inverse function $t\left(\mathbf{R}, \mathbf{R}_{\text{meas.}}\right)$ used throughout this paper.

**Particle Image Velocimetry.** Measurements were performed using a commercial ultra-bright LED pulsed particle image velocimetry (PIV) system (LED Pulsing System; ILAS1500 GmbH), with polystyrene particles as flow tracers (mean diameter of 57 µm, density of 1,016 g cm−3), a 5-MP uEye IDS CMOS camera with a 35-mm lens set at a 20-cm working distance, and double 100-µs-long green light pulses shifted 2 ms (overall period set to 10 fps). The LISST-100x Small Volume Flow Through Chamber was precleaned and filled with a 200-L mL (vol/mL) microbeads solution in Milli-Q water. PIV measurements were analyzed using PIVLab software with 3 iterations on each sample (i.e., 3 × 16 × 16 pixels window size). For long time series experiments, the extracted instantaneous velocity fields were further run-averaging over 1-s intervals to evaluate the mean shear rate.

**Field Data.** Field data were obtained as part of a time series survey carried out during the spring to summer transition at Palma Bay at a station located at a depth of 40 m (39.0944° N, 2.6744° E). Herein, presented data correspond to April 23, on which date a _Pseudo-nitzschia_ bloom was observed at depths of 30 to 40 m. Temperature, salinity, and depth were recorded hourly at the sampling site with a moored Aqualog wire-following profiler equipped with an SBE 19plus SeaCAT profiler CTD (conductivity, temperature, and depth). Current velocities were measured with a bottom-mounted Nortek Aquadopp, profiling at 1-m bins.

Water samples for pigment analysis and phytoplankton identification were collected at 5 depths (5, 10, 20, 30, and 40 m) and through the water column with a 2.5-L Niskin bottle. Phytoplankton samples were preserved with Lugol’s iodine solution (1%). The general procedure for identifying and size-quantifying phytoplankton involved sedimentation (24 h) of a sub-sample in a 50-mL settling chamber, and subsequent counting of cells in an appropriate area (60) using a Leica-Leitz DM-IRB inverted microscope. For morphometric analysis, photographs were taken by a SEM videorecorder, and measurements (cell length and width) were made from the digital images using ImageJ software, version 1.51w (https://imagej.nih.gov/ij/) (61). Suspected particle size distribution, including both phytoplankton and other nonalgal components, was measured using the LISST-100x instrument.
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