Topological defects promote layer formation in *Myxococcus xanthus* colonies

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The soil bacterium *Myxococcus xanthus* lives in densely packed groups that form dynamic three-dimensional patterns in response to environmental changes, such as droplet-like fruiting bodies during starvation. The development of these multicellular structures begins with the sequential formation of cell layers in a process that is poorly understood. Here, using confocal three-dimensional imaging, we find that motile, rod-shaped *M. xanthus* cells are densely packed and aligned in each layer, forming an active nematic liquid crystal. Cell alignment is nearly perfect throughout the population except at point defects that carry half-integer topological charge. We observe that new cell layers preferentially form at the position of +1/2 defects, whereas holes preferentially open at −1/2 defects. To explain these findings, we model the bacterial colony as an extensile active nematic fluid with anisotropic friction. In agreement with our experimental measurements, this model predicts an influx of cells towards the +1/2 defects and an outflux of cells from the −1/2 defects. Our results suggest that cell motility and mechanical cell–cell interactions are sufficient to promote the formation of cell layers at topological defects, thereby seeding fruiting bodies in colonies of *M. xanthus*.

The rod-shaped soil bacterium *Myxococcus xanthus* lives in colonies of millions of individual cells that migrate on surfaces. These colonies exhibit a wide range of motility-driven collective behaviours including rippling during predation and fruiting body formation in response to starvation. When nutrients are scarce, individual cells alter their motility to drive the population from a thin sheet coating the underlying substrate to a series of dome-shaped multicellular structures called fruiting bodies. The first step in this process is the sequential formation of cell layers on the top of the original cell monolayer. However, the physical mechanism underlying layer formation remains largely unknown, partly because the rapid development of fruiting bodies makes it difficult to monitor the emergence of new layers in detail.

Here, to overcome this limitation and address how new cell layers emerge from pre-existing ones, we placed *M. xanthus* cells on an agar substrate in the presence of nutrients. In these conditions, the dimensionless inverse Péclet number that characterizes the persistence of cell migration is $P_e^{-1} = 1.28 \pm 0.09$ (Methods and Extended Data Fig. 1). At this value of $P_e^{-1}$, with an average cell density of $\rho_0 = 0.25 \pm 0.04$ cells/µm$^2$ (±s.d.), the colony does not form fruiting bodies but remains as a thin sheet wetting the substrate. Nevertheless, new cell layers and holes spontaneously appear and disappear (Supplementary Videos 1 and 2), allowing us to examine these processes in detail.

To this end, we imaged the colony using a three-dimensional laser scanning confocal microscope with subcellular resolution (Methods). This instrument measures the light reflected from the surface of the sample and does not require fluorescence or cell labeling. By sampling a stack of positions along the microscope’s axis, we simultaneously measured the height and reflectance fields (Fig. 1a–c). Images of the reflectance field revealed that the rod-shaped *M. xanthus* cells are densely packed and aligned with the neighbouring cells; further, cells retain their motility (Fig. 1b and Supplementary Video 1), which is driven by both cell–substrate and cell–cell interactions. The height field showed that the colony organizes into discrete layers rather than a continuous distribution of heights (Fig. 1c). We thresholded the height data to measure the number of layers at every position in the image (Fig. 1d). Colonies of Δ*piliA* mutant cells lacking pili exhibit very similar behaviours (Supplementary Video 3), showing that these extracellular appendages are not required for layer formation or hole opening.

Densely packed, motile, rod-shaped objects can form a state of matter called an active nematic liquid crystal. Active nematics display emergent collective phenomena resulting from the interplay between active stresses (here due to cell motility) and orientational order (here due to mechanical cell–cell interactions). To quantify the orientational order in the *M. xanthus* colony, we used the reflectance field to measure both the degree of alignment, $S(\mathbf{r}, t)$, and the cell orientation angle, $\theta(\mathbf{r}, t)$, as a function of position $\mathbf{r}$ and time $t$ (Fig. 2a,b, Methods and Supplementary Videos 4 and 5). The orientation angle varies smoothly throughout most of the colony, with a correlation length of $\xi_\theta \approx 16.0 \pm 0.5$ µm (Extended Data Fig. 2). However, at a discrete set of points known as topological defects, the orientations meet, and hence, the orientation angle is singular (Fig. 2a–c). Alignment is lost at the defect points, which are often connected by lines with lower alignment than the perfectly ordered background (Fig. 2b). Consistent with the nematic symmetry of cell alignment, we observe point defects with half-integer topological charges of ±1/2 (Fig. 2c–e), +1/2 defects have one axis of symmetry (red segment, Fig. 2d), and −1/2 defects have three axes of symmetry (blue segments, Fig. 2e). As expected in an active nematic fluid, defects are spontaneously created and annihilated either in oppositely charged pairs or individually at the boundaries of a layer or a hole (Supplementary Video 6).

Similar defect dynamics have been found in other systems including vibrated granular rods, mixtures of cytoskeletal filaments and motor proteins, monolayers of mesenchymal and epithelial cells, growing bacterial colonies and colonies of swarming filamentous bacteria. In some cell populations, topological defects influence collective cell motion and can even trigger intracellular responses. In both suspensions of bacteria
swimming in passive liquid crystals and in mesenchymal cell monolayers, cells were found to accumulate around the positive defects and deplete from negative defects. In chaining bacterial biofilms, stress accumulation at −1/2 defects was found to induce a buckling instability leading to sporulation. Further, in epithelial monolayers, increased pressure around +1/2 defects was found to induce cell apoptosis and extrusion. In mesenchymal monolayers, compressive stress around integer defects triggered cell differentiation. Finally, topological defects in the nematic order of supracellular actin fibres have been recently found to organize Hydra morphogenesis.

Here we find that topological defects play an important role in the developmental cycle of M. xanthus: they promote the layering process that leads to fruiting body formation. We observed new layers forming stochastically throughout the colony. However, by identifying and tracking topological defects (Fig. 3a, Supplementary Video 6 and Methods), we found many events in which new cell layers form close to +1/2 defects (Fig. 3b,c) and new holes open close to −1/2 defects (Fig. 3d,e). To quantify this relationship, we measured the distribution of distances between defects of either sign and the locations where new layers and new holes appear. These measurements indicate that it is ~200 times more likely for a new layer to form close to a +1/2 defect than away from it (Fig. 3f), and ~80 times more likely for a new hole to open at a −1/2 defect than away from it (Fig. 3g). Unlike in growing biofilms, where pressure induces layering and verticalization transitions at a critical colony size, we observe local migration-induced layering events independent of the colony size.

To understand the association between topological defects and layering, we modelled the cell colony as a thin film of active nematic fluid (Section I of the Supplementary Note). We describe the cell alignment in terms of the nematic-order-parameter tensor field $Q_{\alpha\beta}(r)$, which is assumed to rapidly relax to its equilibrium configuration. Here, Greek indices indicate spatial components. As cells migrate along the alignment axis, they mechanically interact with neighbouring cells, which induces an anisotropic active stress $\sigma_{\alpha\beta} = \xi Q_{\alpha\beta}$ in the colony. The coefficient $\xi$ is positive (negative) for extensile (contractile) stresses. The distortions in cell alignment found around topological defects induce a non-zero active force density $f_{\alpha} = \partial_{\beta} \sigma_{\alpha\beta}$, which drives cell flows, $\mathbf{v}(r)$. The active forces are balanced by viscous friction forces $\xi_{\alpha\beta} \nu_{\beta}$ arising from cell–substrate interactions:

$$f_{\alpha} = \xi_{\alpha\beta} \nu_{\beta}.$$  \hspace{1cm} (1)

Like the active stresses, we assume that friction forces are anisotropic. Following ref. 31, we account for friction anisotropy via a friction coefficient matrix, $\xi_{\alpha\beta} = \xi \delta_{\alpha\beta} - \epsilon Q_{\alpha\beta}$, where the first term corresponds to isotropic friction with coefficient $\xi$, and $\epsilon$ is the friction anisotropy along the local alignment axis. Previous measurements of the mechanical response of cell–substrate focal adhesions in M. xanthus suggest that friction is smaller along the cell-alignment axis than perpendicular to it, that is, $\epsilon > 0$.

Assuming equilibrium solutions for the order parameter $Q_{\alpha\beta}(r)$ around topological defects, we solved equation (1) to predict the cell flow fields around +1/2 and −1/2 defects (Section II of the Supplementary Note and Supplementary equations (11) and (13)). The results show that +1/2 defects exhibit net self-driven motion along their axis of symmetry (Fig. 4a)—a well-known feature of active nematic fluids. Moreover, cells in front of the defect are aligned perpendicularly to the flow, thus experiencing stronger friction than the cells behind the defect. Therefore, due to the positive friction anisotropy ($\epsilon > 0$), the inflow towards the defect core is stronger than the outflow (Fig. 4a). As a result, cells accumulate at +1/2 defects, and they are eventually extruded vertically to form new cell layers (Fig. 3b,c). For −1/2 defects, anisotropic friction induces a stronger outflow than inflow (Fig. 4b), which explains the opening of holes at these defects (Fig. 3d,e). Were friction isotropic, the velocity field around defects would be symmetric, implying no cell accumulation or depletion and hence no preferential layer formation or hole opening at the defects.
To test our predictions, we measured the cell flows around topological defects by calculating the optical flow from the reflectance movies (Methods). In agreement with our theoretical predictions, the measured flow fields show that +1/2 defects self-propel along their axis; further, there is net inflow towards +1/2 defects and net outflow from −1/2 defects (Fig. 4f and Extended Data Fig. 3). Since the height of the cell colony can vary, the two-dimensional velocity field \( \mathbf{v}(\mathbf{r}) \) can have a non-zero divergence, namely, \( \nabla \cdot \mathbf{v} \neq 0 \) (see equation (3) in the Supplementary Note). In agreement with our predictions, we find that cell accumulation (\( \nabla \cdot \mathbf{v} < 0 \)) occurs mainly in front of +1/2 defects, whereas cell depletion (\( \nabla \cdot \mathbf{v} > 0 \)) is localized in three lobes along the axes of symmetry of −1/2 defects (Extended Data Fig. 4).

To quantitatively assess our model, we fit the theoretical predictions to the measured velocity profile along the midline of the topological defects, that is, \( v_z(x, y = 0) \) (Fig. 4f and Section II.D of the Supplementary Note). The fits yield values for three model parameters: the nematic healing length, \( \xi \), which determines the defect core size; the ratio \( \xi_0/\xi \), between the active stress and the isotropic friction coefficients; and the friction anisotropy \( \epsilon \) (Table I in the Supplementary Note). For both +1/2 and −1/2 defects, we obtain \( \xi \approx 2.5 \mu m \); the defect core is smaller than one cell length, \( l = 7 \mu m \). In addition, we find that the active stress in the bacterial colony is extensile (\( \xi > 0 \)) and the friction anisotropy is positive (\( \epsilon > 0 \)). However, the optimal values of \( \xi_0/\xi \) and \( \epsilon \) are different for the +1/2 and the −1/2 defects. These effective parameter values characterize the average flows around defects, not all of which give rise to new layers or holes. Imposing common fitting parameters for the +1/2 and −1/2 defects, we obtain \( \xi \approx 2.5 \mu m \); the defect core is smaller than one cell length, \( l = 7 \mu m \). In addition, we find that the active stress in the bacterial colony is extensile (\( \xi > 0 \)) and the friction anisotropy is positive (\( \epsilon > 0 \)).

Neglected pressure gradients in equation (1). However, if cells are not readily extruded, the non-uniform flows around a +1/2 defect induce compression in the cell monolayer, leading to a pressure increase in front of the +1/2 defects. The resulting pressure gradient drives an additional flow towards the defect core, which might account for the measured net counterflow. To probe this idea, we generalized our active nematic model to allow for a non-uniform cell density \( \rho(\mathbf{r}) \) and the associated pressure gradients (Section III of the Supplementary Note and Extended Data Fig. 6). Then, the force balance can be expressed as

\[
\xi_{\alpha\beta} \partial_\beta P(\rho) = -\partial_\alpha P(\rho) + f_{\alpha}^L,
\]

and we assume a linear equation of state \( P(\rho) = B[\rho - \rho_0]/\rho_0 + P(\rho_0) \), where \( B \) is the bulk modulus of the monolayer. With additional assumptions about the rate of cell extrusion (Section III of the Supplementary Note), this extended model produces a counterflow, as shown by the fit of the predicted midline velocity (yellow curve in Fig. 4f and Section III.C of the Supplementary Note). Gaining further insights into the physical origin of the counterflow will require future experiments that can accurately measure the cell density and mechanical stress around the defects, for example, employing cell segmentation algorithms and traction force microscopy, respectively.

In summary, a dense monolayer of \( M. xanthus \) cells migrating on a surface forms an active nematic liquid crystal. Spontaneously created topological defects of the liquid-crystalline alignment promote the formation of new cell layers and holes in the bacterial colony. The localization of these layering events at the topological defects results from the anisotropic friction that the cells experience as they migrate. Thus, we propose that cell motility and mechanical interactions between the cells are sufficient to drive the formation of multilayered structures. We have performed our experiments in the presence of nutrients. However, the layering mechanism that we have uncovered is a generic feature of dense active nematics with anisotropic friction on a substrate. Therefore, we expect that the same mechanism is responsible for layer formation when nutrients are scarce. Under starvation, \( M. xanthus \) cells alter their motility to drive the aggregation of the colony into dense cell monolayers, from which fruiting bodies form. Future work will be needed to connect such starvation-induced changes in motility to the full development of stable fruiting bodies.

Finally, the formation of multilayered cell structures from an initial cell monolayer is not unique to \( M. xanthus \); it occurs in biofilms...
Fig. 4 | Asymmetric cell flows around topological defects explain the formation of new layers and holes. The defect schematics show the order parameter (colour map) along with director-field lines. **a–d.** Theoretically predicted (a,b) and experimentally measured (c,d) flow fields around +1/2 (a,c) and −1/2 (b,d) topological defects. Colour maps indicate the speed and white arrows indicate the velocity. Defect cores are located at the origin of coordinates. Averages are taken over a total of 7,896 frames from 96 tracks of +1/2 defects and 7,096 frames from 144 tracks of −1/2 defects across eight replicate experiments (Methods). The average flow fields measured in individual replicate experiments are shown in Extended Data Fig. 8. e. Maximal net inflow of cells at +1/2 defects (V_{net}^{max} > 0, red), and net outflow from −1/2 defects (V_{net}^{max} < 0, blue), as seen in the coarsely sampled predicted flow fields around defects shown on the right. V_{net}(R), as defined in Supplementary equation (21) in the Supplementary Note, is the average net inflow velocity through a circle of radius R centred at a defect (Extended Data Fig. 3). V_{net}^{max} is the maximum of V_{net}(R) in the experiments, which occurs at R = 8 μm for the +1/2 defects and at R = 6 μm for the −1/2 defects (dashed green circles in a–d). The theoretical V_{net}^{max}(R) is given in Supplementary equation (18) in the Supplementary Note. The corresponding error bars are obtained by propagation of errors in the parameter values (Table I in the Supplementary Note).

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Methods
Cell culture. *M. xanthus* cells of the wild-type strain DK1622 are grown in CTTYE (1% casitone, 10 mM Tris–HCl (pH 7.6), 1 mM KH$_2$PO$_4$, and 8 mM MgSO$_4$) overnight to a concentration corresponding to a range of OD$_{600}$ = 0.4–0.8, where OD$_{600}$ is the optical density at a wavelength of 600 nm. This culture is then concentrated and resuspended into fresh CTTYE to an OD$_{600}$ value of 2. Then, 5 µl of the concentrated culture is placed onto a CTTYE pad with 1.5% agarose and allowed to dry until no liquid is visible on the surface of the agarose gel. Next, the sample is incubated for 2 h before imaging.

Imaging. We imaged our samples using a Keyence VK-X1000 microscope. The microscope works by scanning a laser across the surface of the sample and measuring the reflectance of the laser light from the surface. The reflectance of this sample is measured at multiple heights. Then, a height field of the sample is obtained by finding—for each pixel—the height at which the reflectance is maximum. Finally, the reflectance field at the height of this maximum reflectance gives clear and in-focus images of the top of the sample (Fig. 1a).

Imaging was done at a frame rate between 4.8 and 7.2 min$^{-1}$ from 1 to 3 h. During this time, the colony does not grow substantially. At the temperature of the experiments, namely, $T = 22$°C, the cell doubling time is $\sim$10 h (ref. 10). The entire cell colony is over 1 cm in diameter; our field of view is a region of $\sim$150 × 100 µm.

The data in this manuscript come from eight replicate experiments.

Cell motility measurements and state of the colony. *M. xanthus* colonies undergo a transition from the non-aggregated state (in which the colony forms a thin film on the agar substrate) to an aggregated state (which leads to fruiting body formation). A recent study has shown that this transition is controlled by a dimensionless number known as the inverse rotational Péclet number

$$P_e^{-1} = \frac{\kappa_{cell}}{v_c} (D_r + 2f_{rev}),$$

which combines the effects of the cells’ self-propulsion speed $v_c$, their rotational diffusion coefficient $D_r$, and the average rate of velocity reversals $f_{rev}$ (ref. 2). The cell length, $\kappa_{cell} = 2.5$ µm, denotes the effective average cell size.

To study the layer formation, we perform experiments in the presence of nutrients, which maintains the cell colony in the regime of cell migration parameters corresponding to the non-aggregated state. In this state, fruiting bodies do not form, but new cell layers and holes continuously appear and disappear, providing us with many layer-formation and hole-opening events that allow us to study these processes in detail. If we performed the experiments under starvation conditions, the colony would transition into the aggregated state and fruiting bodies would rapidly form, preventing us from imaging the layer-formation process in sufficient detail.

To check that our experiments lie within the appropriate parameter regime, we tracked cells moving at a low cell density corresponding to OD$_{600}$ = 0.1, and we measured the probability distribution both of cell speeds and of the times between velocity-reversal events (Extended Data Fig. 1). We find that cells move with an average speed of $v_s = 0.922$ ± 0.009 mm min$^{-1}$ and an average reversal rate of $f_{rev} = 0.172 ± 0.002$ min$^{-1}$. The value of the rotational diffusion coefficient, $D_r = 0.127$ min$^{-1}$, was obtained from ref. 6. Using these motility parameter values, we obtain $P_e = 1.28$ ± 0.09, which lies well within the non-aggregated state in the phase diagram obtained in ref. 2.

Nematic order. To characterize the nematic order in the cell colony, we measure the orientation angle field $\theta(x, t)$ and the nematic order parameter strength field $S(x, t)$ from the laser brightness images of the colony. Following ref. 5, we first sharpen the laser-light signal by means of a high-pass filter. Then, we compute the gradient of the resulting brightness field $I_x$ where the indices $ij$ indicate the image pixels. We smooth the brightness gradient by means of a Gaussian filter with standard deviation $\sigma = 1.7$ µm. Then, for each pixel, we build the so-called structure tensor

$$H_{ij} = \begin{pmatrix} (\partial_x I_x)(\partial_x I_x) & (\partial_x I_x)(\partial_y I_y) \\ (\partial_y I_y)(\partial_x I_x) & (\partial_y I_y)(\partial_y I_y) \end{pmatrix},$$

and we obtain its eigenvalues and eigenvectors. The eigenvector associated with the smallest eigenvalue gives the local direction of the smallest brightness gradient, which can be identified with the angle field $\theta(x, t)$ of cell orientation.

From the measured angle field $\theta(x, t)$, we obtain the order parameter strength field $S(x, t)$

$$S = \langle 2(\cos \theta - \bar{\theta}_g)^2 \rangle,$$

where $\bar{\theta}_g = \langle \theta(x, t) \rangle$ is the average orientation angle within a disk of radius $R = 1$ µm centred at pixel $(i, j)$. $S$ is almost 1 over the majority of the system, indicating almost perfect cell alignment, but it approaches 0 at the defects, where cell alignment is lost and the angle field becomes singular (Fig. 2b).

Following ref. 2, we measure the following spatial correlation function of the nematic orientation angle:

$$C_s(r) = \langle \mathbf{n}_0(\mathbf{r}) \cdot \mathbf{n}_0(0) \rangle,$$

where $\mathbf{n}_0 = (\cos 2\theta, \sin 2\theta)$. The average is taken from over 10,000 randomly selected pairs of points from every frame of every experiment. We fit an exponential decay with an offset, $C_s(r) = A e^{-r^2/\ell^2} + B$, to the measured correlation function (Extended Data Fig. 2a) and obtain the two fitting constants $A$ and $B$, and a nematic correlation length of $\ell = 16.0 ± 0.5$ µm.

Finally, to measure the equilibrium value of the order parameter strength, $S_e$ (Supplementary Text), we obtain the average $S_e = \langle S(x, t) \rangle$ over regions of the cell colony without defects. Specifically, we exclude circular regions with radius $R_{agg} = 16$ µm ± $\ell$, around the topological defects (see below for a description of the defect detection method). In addition, we also exclude the holes in the cell colony, that is, regions without cells. Using this procedure, we obtain $S_e = 0.992 ± 0.003$.

Defect detection and tracking. We identify and track defects by finding local minima of the nematic order parameter strength $S(x, t)$ with $S < 0.7$. Often, several pixels around these local minima have the same value of $S$. Thus, we identify the defect core with the centroid of all the pixels that share the local minimum value of $S$.

For each point identified as a potential defect core, we calculate the topological charge $q$ by applying its definition:

$$q = \frac{1}{2\pi} \oint \frac{d\theta}{c},$$

where $C$ is a circular circuit of radius $r = 1$ µm around the defect core. We ignore the candidate points with $q = 0$, as well as the ones that fall in holes in the cell colony.

For each defect, we identify its axes of symmetry as recently proposed in ref. 6. Specifically, for defects with topological charge of $+1/2$, the defect axis is given by $p_x = \partial_x Q_{00}/\partial_x Q_{20}$, where $Q_{00}$ is the nematic order parameter tensor with components $Q_{00} = S_2 n_x n_y - Q_{yy}$, where $Q = \{\cos \theta, \sin \theta\}$ is the director field. Similarly, for defects with topological charge of $-1/2$, we first define the opposite nematic angle $\theta' = -\theta$ and we obtain the corresponding $Q'$ tensor. From this, we obtain $p_x' = \partial_x Q'_{00}/\partial_x Q'_{20}$. Finally, defining $p_x = (\sin \psi, \cos \psi)$, one symmetry axis of a $-1/2$ defect is then defined by the three-fold symmetry of $-1/2$ defects.

Finally, we track the defects over time by finding the closest defects with the same charge in consecutive image frames. We ignore the defects that last for less than ten frames (~2 min). Across eight replicate experiments, we found 96 defects with a topological charge of $+1/2$ and 144 defects with $q = -1/2$. All together, the $+1/2$ defect tracks consist of a total of 7,096 frames, which are included in the averaged data in Figs. 2d and 4c. Respectively, a total of 7,096 frames of $-1/2$ defects are included in the averaged data in Figs. 2e and 4d.

Cell flow measurements. We measure the cell flows by applying an optical flow algorithm on the laser-brightness images of the cell colony. The optical flow algorithm is based on the Lucas–Kanade derivative of the Gaussian method12. By comparing the independent velocity measurements from a small number of manual cell trackings to the optical flow measurements, we find a calibration parameter to convert the optical flow to a cell velocity field within the bacterial colony.

Finally, we measure the spatial correlation function of the velocity field:

$$C_v(r) = \langle \mathbf{v}(\mathbf{r}) \cdot \mathbf{v}(0) \rangle,$$

As for the nematic correlation function in equation (6), the average is taken from over 10,000 randomly selected pairs of points from every frame of every experiment. We fit an exponential decay, $C_v(r) = D e^{-r^2/\gamma}$, to the measured correlation function (Extended Data Fig. 2b) and obtain the fitting constant $D$ and a velocity correlation length of $\gamma = 3.9 ± 0.1$ µm.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Source data are provided with this paper. All other data that support the plots and findings of this study are available from the authors upon request.

Code availability
All codes are available from the authors upon request.

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Author contributions
K.C. performed the experiments and analysed the data. R.A. developed the theory and fitted the predictions to the experimental data. All authors interpreted the results and designed the experiments. N.S.W. and J.W.S. supervised the study. K.C. and R.A. wrote the manuscript with input from all the authors.

Competing interests
The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41567-020-01056-4.
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Extended Data Fig. 1 | Statistics of *M. xanthus* motility. Histograms of the cell speed (a) and time between velocity reversals (b) of cells migrating in a low-cell-density environment.
Extended Data Fig. 2 | Correlation functions. Spatial correlation functions of (a) the modified director field $\hat{n}_\theta = (\cos(2\theta), \sin(2\theta))$, and (b) of the velocity field $v$. Error bars are s.e.m. By fitting exponential decays, we obtain the nematic and the velocity correlation lengths: $\ell_n = 16.0 \pm 0.5 \, \mu m$ and $\ell_v = 3.9 \pm 0.1 \, \mu m$, respectively.
Extended Data Fig. 3 | Net inflow around topological defects. $V_{\text{net}}$, defined in Supplementary Eq. 15 in the Supplementary Note, is the average net inflow velocity through a circumference of radius $R$ centered at a topological defect. Points are experimental data obtained from the average flow fields in Fig. 4c,d. Error bars (s.e.m) are barely visible because they are smaller than the point size. Solid curves are the theoretical predictions given in Supplementary Eq. 18 in the Supplementary Note, evaluated using the parameter values in Table I in the Supplementary Note. Vertical dashed lines indicate the radii at which the experimental net inflow magnitude is maximal. This maximal net inflow is presented in Fig. 4e.
Extended Data Fig. 4 | Divergence of the flow field. Theoretically predicted (a, b) and experimentally measured (c, d) divergence fields around topological defects. The defect schematics show the order parameter (color map) and a few director-field lines. The divergence of the two-dimensional flow field, $\nabla \cdot \mathbf{v}$, shows pronounced cell accumulation ($\nabla \cdot \mathbf{v} < 0$, purple) in front of $+1/2$ defects, and cell depletion ($\nabla \cdot \mathbf{v} < 0$, green) in three lobes along the axes of symmetry of $-1/2$ defects. The parameter values used to plot panels a and b are listed in Table I in the Supplementary Note.
Extended Data Fig. 5 | Simultaneous fits of the velocity profiles of $+\frac{1}{2}$ and $-\frac{1}{2}$ defects. The black (grey) data points and the red (blue) curve are the experimentally measured and the fitted midline velocity profile of $+\frac{1}{2}$ ($-\frac{1}{2}$) defects, respectively. These simultaneous fits to $+\frac{1}{2}$ and $-\frac{1}{2}$ defects, with the common set of parameter values given in Table II in the Supplementary Note, are poorer than the separate fits to the $+\frac{1}{2}$ and $-\frac{1}{2}$ defects shown in Fig. 4f.
Extended Data Fig. 6 | Predicted cell density profile along the midline of +1/2 defects. The total cell density is $\rho(x, 0) = \rho_0 + \delta\rho(x, 0)$, where $\delta\rho(x, 0)$ is given by Supplementary Eq. 31 in the Supplementary Note, with $v_0^2(x, 0)$ obtained from the fits (parameter values in Table III in the Supplementary Note), and $J=10^{-4} \mu m^{-1}$. 
Extended Data Fig. 7 | Probability distributions of the distance between defects and new layers (a), and between defects and new holes (b). In Fig. 3f,g, we normalized these distributions with the distribution $p_x(r)$ of distances between defects and randomly selected points within the monolayer (excluding holes). Here, as commonly done for radial distribution functions $g(r)$, we normalized the distributions by the area of an annulus of width $dr$ (the histogram bin size), with $A$ the area of the field of view. Both normalizations give similar results. Errors are s.d.
Extended Data Fig. 8 | Average flow field around defects separately measured in each of the 8 replicate experiments. Each of the separate averages includes a different number of defect frames. The total averages in Fig. 4c,d include all the defect frames across the 8 replicate experiments.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

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Data collection: Custom C++ code using Dynamic-link library (DLL) files provided with the Keyence microscope VK 1000x.

Data analysis: Custom Matlab code to perform all the data analysis. GnuPlot to fit the theoretical expressions to the experimental measurements of the flow field.

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| Sample size | Eight time lapses were collected between one and three hours in length from four different samples, limited by difficulty of sample preparation. |
| Data exclusions | No data was excluded. |
| Replication | All measured quantities from all eight datasets gave similar results. |
| Randomization | The experiments were not separated into separate experimental groups at any point. |
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