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The mechanisms of spatial pattern transition in motile bacterial collectives

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To understand how individual behaviors contribute to collective actions across scales—ranging from molecular interactions to animal populations—it is essential to identify the fundamental rules that govern these interactions. *Myxococcus xanthus*, a bacterial predator, can produce very large multicellular patterns, transitioning from a swarming behavior to a rippling behavior due to changes in the local environment within and around prey colonies. Through a combination of high-resolution microscopy and theoretical analysis, we demonstrate that this transition can be explained by two simple properties, local cellular alignment guided by an extracellular matrix and the ability of cells to resolve congestion by reversing. Furthermore, we show that a tunable refractory period in the reversal control system enables a wide range of collective adaptations, allowing cells to synchronize during rippling and relieve congestion during swarming. Remarkably, our models suggest that these transitions can occur without genetic regulation changes, and result in stable spatial domains that facilitate local differentiation.

In biology, remarkable patterns emerge from collective motion across scales—from migrating cells to flocks of sheep—shaping how communities self-organize. Understanding how such patterns arise from interactions between individual agents remains a central challenge¹. In wild animals and humans, deciphering the rules of interaction underlying spatial organization is difficult because individuals are hard to track and experimental perturbations are rarely possible². In this respect, bacterial model systems are powerful: they are amenable to both high-throughput tracking and genetic manipulation. Here, we use the social bacterium *Myxococcus xanthus* (*M. xanthus*), a predatory species, to investigate the molecular and cellular basis of multicellular pattern transitions during predation of prey colonies.

When a *M. xanthus* colony encounters a prey colony of *Escherichia coli* (*E. coli*), predatory cells spread outward from the initial inoculum—a process referred to as swarming³. Typically, this behavior involves

the concerted movement of large groups of cells that tend to follow self-deposited trails. This process is distinct from swarming referring to flagellated bacteria: it relies on two motility systems—retractile type IV pili and A-motility—and on a self-produced exopolysaccharide (EPS). The EPS is deposited as cells move, forming so-called "slime trails" that guide collective migration^{4,5}. The extracellular matrix (ECM) contain other polymeric substances, such as lipids and biosurfactants, and prey-derived matrix. The EPS component is absolutely required for motility because the Type IV pili, assembled at the cell poles, binds specifically to EPS trails and retracts, allowing movement along these trails⁶. A-motility, based on focal adhesion complexes, allows individual cells to detach from the groups and explore the prey environment, acting as scout cells⁷. The two motility machineries can disassemble from one pole and reassemble at the opposite pole, allowing cells to rapidly change direction—a process known as reversal. Mutants unable to reverse show severe swarming defects^{8,9}.

Following prey consumption and the onset of starvation, cells within the prey area undergo a striking transition: they organize into concerted multicellular waves ("ripples") that propagate across the prey surface over long distances¹⁰ (Fig. 1a,b and Movie S1). Outside the prey area, cells continue to swarm, and the two multicellular states are sharply separated by an interface defined by the prey boundary. Evidence suggests that this transition is triggered by changes in the local environment, most notably the presence of a prey-derived matrix. Indeed, deposition of *M. xanthus* cells onto purified prey cell wall fragments is sufficient to induce rippling, presumably through persistent cell alignment driven by a yet unknown mechanism³.

These distinct multicellular states—swarming structures and rippling waves—have inspired a wide range of theoretical models aimed at uncovering the underlying rules of collective behavior in *M. xanthus*. Among these, rippling has been the most extensively studied, particularly through one-dimensional continuum approaches based on Partial Differential Equations (PDE). These models represent populations as left- and right-moving cell densities and show that ripples can emerge from density-dependent reversals combined with a fixed refractory period (RP)—defined as the delay following a reversal during which a cell cannot reverse again^{11,12,13,14,15}. Beyond reproducing wave-like behavior, these models provide analytical insight into the conditions for ripple emergence and stability. In particular, two ingredients are essential to sustain ripples: a sufficiently strong nonlinearity in the reversal response to signaling and a sufficiently long RP^{11,14,15}.

Alternatively, agent-based models have also been developed to explore how ripples self-organize in a two-dimensional space. In addition to the reversals and their RP, ripple formation was shown to require local nematic alignment between neighboring cells^{16,14}, consistent with the strong cellular orientation observed experimentally in rippling fields. Such alignment constrains the system to a quasi-one-dimensional geometry in which cells remain globally aligned, successfully reproducing the accordion-like motion seen in experiments, where cells reverse upon collision of counter-propagating waves¹⁷.

Similarly, swarming has been investigated using two-dimensional agent-based models focusing on mechanical interactions between cells. These models incorporate reversals, steric repulsion, and slime trail-following, allowing agents to align with paths previously traveled by others^{8,18}. Trail-following, motivated by experimental observations of EPS-mediated alignment, was shown to be crucial for maintaining cohesive groups and forming the structures observed during swarming.

Together, these studies propose several mechanisms regulating *M. xanthus* behavior. In both swarming

and rippling, reversal control is central, but each model assumes distinct reversal dynamics. Depending on the swarming model, reversals are periodic or stochastic, whereas in rippling, reversals are triggered by a signal known as the C-signal, thought to activate reversals upon cell-cell collisions^{19,20}. However, the signaling role of the C-signal remains debated: its associated protein, CsgA, is a cardiolipin phospholipase involved in lipid metabolism during starvation, questioning its function as a surface-exposed mediator of contact-dependent communication²¹.

Although the exact cues triggering reversals remain unclear, the genetic pathway controlling them is well established, particularly the mechanism underlying the RP. In *M. xanthus*, front-rear polarity is governed by MglA, a small GTPase that cycles between an active GTP-bound and an inactive GDP-bound state. In its active form, MglA localizes at the leading pole, where it recruits motility complexes to drive movement. At the lagging pole, the GAP MglB hydrolyzes MglA-GTP to GDP, thereby inactivating it. Reversals occur when the polarity of MglA-GTP switches, a process regulated by the Frz signaling pathway²².

Reversal initiation involves activation of the FrzCD receptor, leading FrzE kinase to phosphorylate two response regulators, FrzX and FrzZ^{23,24} (Supplementary Text Fig. 7). Phosphorylated FrzX (FrzX~P) acts at the lagging pole, presumably by preventing MglB from hydrolyzing MglA-GTP. Phosphorylated FrzZ (FrzZ~P) acts at the leading pole, where it likely promotes detachment of MglA-GTP, thereby increasing the cytoplasmic pool available for relocation to the lagging pole. Once a reversal has occurred, subsequent Frz activation can only trigger another reversal after the RP has elapsed.

At the molecular level, the RP is defined by the dynamics of RomR and FrzZ~P. RomR plays a dual role: following a reversal, it recruits MglA-GTP to the new leading pole, then gradually relocates to the lagging pole through interaction with MglB, facilitating dissociation of the MglA-MglB complex^{24,25,26}. The rate of RomR relocation determines RP length because a critical concentration at the lagging pole is required to recruit MglA during the next reversal. The Frz system can therefore operate in two regimes (see Supplementary Text Fig. 8):

- Low-signaling regime: When [FrzX~P] and [FrzZ~P] are low, RP duration is set by RomR relocation dynamics. A reversal can occur once RomR is relocated at the lagging pole and [FrzX~P] transiently increases following FrzCD activation.
- High-signaling regime: When FrzCD activation is strong, FrzX~P outcompetes MglB, diminishing its inhibitory effect, while FrzZ~P detaches MglA-GTP from the leading pole. This increases intracellular [MglA-GTP] and shortens the RP in a FrzZ~P-dependent manner.

In summary, existing models describe rippling and swarming patterns using distinct rule sets—differing in reversal dynamics, refractory periods, and cell-cell interactions—necessitating two separate frameworks to reproduce adjacent patterns. Recent understanding of the reversal system, particularly the regulation of RP duration, calls for revisiting these assumptions. In this study, we combine an experimental system that captures single-cell behaviors in adjacent swarming and rippling fields after predation, together with a pair of models, to explore the mechanisms underlying the co-existence of both patterns (Fig. 1a,b). Using one-dimensional mathematical modeling, we first show that modulation of the RP facilitates rippling emergence. We then identify a unique signal of reversal—triggered by cell congestion—which is common to both rippling and swarming fields. Finally, by implementing a two-dimensional agent-based

119 model governed by the same rules in both fields, except for cell alignment, we reproduce the side-by-side
120 dynamics of rippling and swarming. Our results demonstrate that a common reversal mechanism, com-
121 bined with field-specific alignment, is sufficient to explain the emergence, stability, and coexistence of
122 both multicellular behaviors in *M. xanthus*.

123 124 **Rippling and swarming exhibit distinct spatial alignment and reversal dynamics**

125 To elucidate how cellular rules differ between swarming and rippling behaviors, we first analysed the
126 levels of cell alignment in each of these two fields by quantifying changes in local nematic order – a metric
127 between 0 (disorder) and 1 (perfect alignment) commonly used in active matter physics to describe how
128 aligned elongated particles (here, cells) are with respect to one another, irrespective of their head or tail
129 orientation²⁷ (see Methods). In swarming fields, cell alignment extended only over short ranges, drop-
130 ping rapidly as distance increases (Fig. 1c). By contrast, in rippling fields, and consistent with previous
131 works^{28,17}, we found that cells were characterised by persistent alignment over longer distances.

132 We next asked whether cell reversals are required to establish alignments observed in rippling and
133 swarming fields. To test this, we used the *frzE* mutant, which is strongly defective in reversals²⁹, hence
134 unable to form rippling waves (fig. S1), while producing EPS trails. We found that *frzE* cells still become
135 highly aligned upon entering the prey area (fig. S2). Outside the prey area, we observe that *frzE* still
136 forms coherently moving flocks, consistent with previous observations⁹. Thus, reversals are not required
137 for alignment in either field (compare fig. S2c and Fig. 1c). In both cases, alignment likely results from
138 interactions with the ECM, cell-cell interactions and capillary forces as recently demonstrated³⁰.

139 Analysis of single-cell trajectories in rippling fields, visualized in space-time kymographs, revealed the
140 characteristic zigzag motion of the cells, with reversal events occurring predominantly at sites of wave
141 collision and occasional instances of cells crossing wave fronts, consistent with previous studies^{19,28,17} (Fig.
142 1d, movie S2). Importantly, multiple rapid reversals were also observed during wave collisions, a phe-
143 nomenon consistent with the shortening of the RP described in Guzzo et al.²⁴ at the single cell level. We
144 next analysed the dynamics of reversals in each of the rippling and swarming fields. To detect reversals
145 with precision, we designed an algorithm that accurately identifies reversals from high-time-resolution
146 phase-contrast time-lapse movies of bacterial populations (see Materials and Methods and Supplemen-
147 tary Text, Section 2). Measuring the distribution of time intervals between two consecutive reversals
148 (TBR) we found that rippling fields were characterised by a bimodal distribution, where the first mode
149 corresponds mainly to cells reversing multiple times during the collision of two opposite waves and the
150 second mode corresponds to cells that take part in a density wave, and reverse against the opposite wave
151 (Fig. 1d,e). This distribution is consistent with earlier trends on much lower cell numbers²⁸ (N=20). In
152 contrast, the second mode is absent in the TBR distribution in swarming fields which appears unimodal
153 (Fig. 1f). Thus, tracking individual cells in each of the swarming and rippling fields reveals different
154 signatures (alignment and TBR) that should be captured in any simulation attempting to recapitulate
155 these patterns. Altogether our results show that rippling and swarming exhibit distinct spatio-temporal
156 dynamics which may explain the observed differences in collective patterns.

157 158 **A new theoretical framework for the role of reversal regulation in pattern formation**

159 We next modeled the emergence of rippling waves. Previous one-dimensional PDE models had already

reproduced rippling and identified reversals as essential. Specifically, they showed that a RP was crucial for pattern formation.^{11,31,12,32,33,16,14,15}. In these models, the duration of the RP is encoded by a fixed parameter, preventing cells to reverse multiple times during wave collisions. This does not match with the individual trajectories observed in rippling fields (Fig. 1d), nor with the TBR bimodal distribution with a large fraction of small time intervals (Fig. 1e). Noticeably, the modulation of the RP²⁴, instead offers a mechanism consistent with the rapid reversals observed during wave collisions.

This dual operation of the Frz system raises the question of how each signaling regime contributes to the emergence of collective patterns. To address this, we constructed a mathematical model that incorporates both signaling regimes. We updated an age-structured model¹⁴ based on PDE (see Fig. 2a) describing the evolution of a bacterial density in time (t) in a one-dimensional space (x) (cross-section of the rippling field), which is splitted between right-moving cells (denoted u^+) and left-moving cells (denoted u^-) at constant speed (v). We also endowed cells with the ability to reverse (i.e. an instantaneous flip in the direction of motion). An additional age variable (r) encodes the time elapsed since the last reversal. It is reset to zero after each reversal. No reversal can occur while r is less than the duration of the RP, denoted by T_{RP} . This is encoded in the equation by a Heaviside function $\mathbf{H}(r - T_{RP})$. The mean time of reversal after the end of the RP is denoted by T_{REV} . Given that downstream from the FrzE kinase, the transduction pathway is branched (see Supplementary Text, Fig. 7) both T_{RP} and T_{REV} were assumed to be modulated by a unique external signal. We encode the two signaling regimes in the model as follows:

- Low-signaling regime: the duration of the RP T_{RP} is constant, while the mean time of reversal T_{REV} is signal-dependent (Fig. 2a, top and Fig. 2b).
- High-signaling regime: T_{RP} is signal-dependent, while T_{REV} is a small constant, so that reversals occur soon after the expiration of T_{RP} (Fig. 2a, bottom and Fig. 2b).

To simulate the triggering of reversals within this 1D geometry, we investigated two distinct signals that have been hypothesized and used in the literature: either the local density (i.e. number of neighbours¹³), or the local directional density (i.e., number of neighbours coming from the opposite direction^{11,31,12,32,33,16,14,15}). Numerical simulations showed that both signals allow the emergence of rippling behavior, provided that signal intensity reaches the high-signaling regime locally at wave collision sites (see Fig. 2c, movie S3).

Next, we aimed to understand the conditions (the model parameter range) under which pattern formation occurs. We conducted a linear stability analysis of the system of PDE (Fig. 2a) by introducing fluctuations around the spatially homogeneous steady-state. We studied the time evolution of these fluctuations (via their Fourier modes) which determines whether they decay (stability), or whether they grow (instability) leading to pattern emergence. The Fourier modes are taken with respect to the spatial variable (x), since the problem is translationally invariant in that direction. The profiles of the fluctuations in the age variable (r), depending on the Fourier mode frequency (ξ), are solution to a renewal equation which recapitulates the reversal dynamics (aging of the variable r , and reset $r = 0$ at reversal). The analysis is standard, but should be handled with care, especially for the modulation of the RP which involves Dirac masses when differentiating the Heaviside function $\mathbf{H}(r - T_{RP}(\rho))$ with respect to the signal intensity ρ (see Supplementary Text, Section 4.2).

The linear stability analysis entails computing the complex eigenvalue $\Lambda(\xi)$ for each mode of the fluctuation at spatial frequency ξ . Such eigenvalue encodes the decay/growth rate of this mode via the sign

of its real part, that is either $\text{Re}(\Lambda(\xi)) < 0$ (decrease of the fluctuation at frequency ξ), or $\text{Re}(\Lambda(\xi)) > 0$ (increase of the fluctuation at frequency ξ). The possible emergence of a pattern is characterized by the positivity of the maximum value of these real parts over all possible frequencies, denoted by $\text{Re}(\Lambda)$. Nondimensionalizing the model reveals that Λ depends only on two numbers: 1) the ratio between the two characteristic times $S = T_{RP}(\rho)/T_{REV}(\rho)$ and 2) the coupling intensity C representing the sensitivity to the signal (ρ) in logarithmic scale, $C = \frac{-\partial \log(T_{REV} + T_{RP})}{\partial \log \rho}$. The latter can be expressed as a function of S for an explicit model of signal integration via the choice of the functions T_{RP} and T_{REV} . We opted for a linear relationship between $T_{REV}^{-1}(\rho)$ and ρ (i.e. T_{REV} is inversely proportional to ρ) in low-signaling regime, and an inversely proportional relationship between T_{RP} and ρ in high-signaling regime (see Fig. 2b). This modeling choice is guided both by biological observations (as explained above) and mathematical tractability for a better interpretability. The values of $\text{Re}(\Lambda)$ plotted as a function of S for all values of ρ are shown in Fig. 2d for the directional density signal and Supplementary Text, Fig. 13b for the local density signal.

Our mathematical analysis reveals that, for both signals, patterns are absent (for a local density signal) or they have slow temporal growth (for directional density signal) in the low-signaling regime. In contrast, counter-propagating waves can emerge in the high-signaling regime, at least for a value of S sufficiently large. The choice of the signal (density or directional density) has no qualitative but only quantitative impact on pattern formation. These results reveal two important conclusions. First, the directional density signal, compared to the local signal, facilitates the formation of counter-propagating waves thanks to a wider parameter range for S and a faster emergence of the waves. Second, in the high-signaling regime, the ability to shorten the TBR—via RP modulation—facilitates synchronisation between individual bacteria which can generate counter-propagating collective waves. This highlights RP modulation as an essential ingredient for pattern formation, which can arise when the RP is sufficiently long relative to the mean time of reversal.

We tested the robustness of this conclusion on previously published phase-structured models (see Supplementary Text, Section 4.4.1) that describe the reversal process as an oscillator^{11,16}. In this model, reversal occurs when the cell has completed a cycle, composed of a fixed RP followed by a sensitive period subject to modulation. We extended the model to allow for the modulation of the RP. Then, we performed the linear stability analysis of this phase-structured model as we had done for the age-structured model (taking into account that the former is more singular than the latter). We showed that, all parameters being equal, patterns emerge faster and more effectively when the RP is modulated (see Supplementary Text, section 4.4.3), consistent with the results of the age-structure model.

Finally, we explored a wider class of signal integration models, via the choice of the functions T_{RP} and T_{REV} , and subsequently the reduced parameters S and C . Unsurprisingly, a more nonlinear relationship yields a larger value of C which facilitates the emergence of pattern even in the low-signalling regime, consistently with Manhart¹⁵. However, increasing nonlinearity in the model of signal integration reduces the range of signal levels for which the system is sensitive to fluctuations. The switch from low- to high-signalling regime enables the emergence of patterns over a much broader range of signalling levels.

In conclusion, all available theoretical frameworks show that the modulation of the RP reported in Guzzo et al.²⁴ provides flexibility in how individual cells integrate the signal and reverse accordingly, leading to synchronization at the population scale.

242 Reversals are correlated with frustrations resulting from spatial constraints in swarming 243 and rippling fields

244 The one-dimensional rippling model described above relied on density-based signals to regulate reversals,
245 as proposed in the literature (local or directional density). We therefore tested whether such signals could
246 account for reversal dynamics in our experimental data. In rippling fields, we found that reversals are
247 slightly correlated with directional density but not with local density (fig. S3a). This is consistent with
248 our 1D age-structured model, which shows that although both signals can sustain pattern formation,
249 directional density has a quantitatively stronger impact than local density on wave emergence (see previ-
250 ous paragraph). In swarming fields, however, no clear positive correlation with either density signal was
251 observed (fig. S3b; we even observed a negative correlation for the local density signal).

252 These observations prompted us to search for a more general reversal signal, resulting from local in-
253 teractions which would be common to rippling and swarming fields. We reasoned that cellular congestion
254 (i.e. traffic jams) rather than instantaneous collisions or immediate density could be activating reversals.
255 To analyse how congestion affect cellular collectives over large distances, we considered previous studies
256 on crowd dynamics inspired by granular flows where contacts between agents generate a pressure net-
257 work which globally modifies the flow. Since the pressure network can have large-scale components^{34,35},
258 deciphering the individual dynamics from the macroscopic configuration is a delicate issue a priori. Never-
259 theless, a meaningful indicator of jamming: the so-called frustration index, f , can be defined a posteriori
260 at the individual level. This index measures the deviation from the target velocity of each agent resulting
261 from the dynamics of others. It is a positive number taking values between 0 and 2, vanishing if the active
262 agent actually moves with its target velocity, or being large when it strongly deviates in jammed areas
263 (see Fig. 3a and Supplementary Text, Section 5).

264 Here, we prescribed the target velocity as the velocity aligned with the cell body with constant speed.
265 *M. xanthus* cells should deviate their trajectories, slow down, or both, when they experience congestion.
266 We measured these deviations in each trajectory, averaging them over a few time frames to capture a
267 smooth signal, in both swarming and rippling fields (Fig. 3a-c) and plotted the frustration distributions in
268 Fig. 3d. We observe that cells in swarming experience relatively higher frustration over their lifetime, as
269 indicated by the right shift of the distribution compared to rippling. Moreover, the swarming distribution
270 exhibits low skewness and a larger variance around the mean at 0.68, suggesting a more continuous range
271 of frustration values. In contrast, the rippling distribution is negatively skewed and peaks sharply around
272 0.48, indicating more homogeneous frustrations. These observations suggest that swarming entails higher
273 congestion as it involves a broader and more heterogeneous experience of frustration across time, whereas
274 frustration events in rippling are peaked, which likely correspond to wavefront collisions.

275 We next investigated whether reversals preferentially occur when a cell experiences high levels of
276 frustration along its trajectory. To account for variability between individual cells, we compared the
277 frustration at every recorded reversal with the distribution of all frustration values for each individual
278 trajectory. This approach highlights instances of unusually high frustration for each cell, rather than
279 relying on a fixed global threshold, and enables a unified comparison across cells and conditions (rippling
280 vs. swarming).

281 We found that $(51.0 \pm 2.5) \%$ of the reversals occurred within the highest frustration quartile in the

282 rippling fields (Fig. 3e, fig. S4). The same analysis in the swarming field showed a similar relationship,
283 with $(44.0 \pm 2.1)\%$ of the reversals also occurring within the highest frustration quartile. These results
284 suggest that in groups, *M. xanthus* cells may reverse to resolve physical constraints exerted along their
285 trajectory, and which they do regardless of whether they are engaged in swarming or rippling.

286

287 **Simulating multicellular transitions**

288 We then investigated whether a common set of rules, supported by biological knowledge could account
289 for the emergence and coexistence of both rippling and swarming patterns. These rules include ECM
290 changes, frustration-mediated reversals and modulation of the RP.

291 As these mechanisms and behaviors are best captured in a two-dimensional setting, we developed a 2D
292 agent-based model, inspired by Balagam et al.⁸, where each bacterium is modelled as a chain of spheres,
293 connected by springs, capturing both the shape and flexibility of *M. xanthus* cells^{36,37,8} (for detailed
294 information, see the Supplementary Text, Section 6). Cell motility was modeled by cellular traction at
295 the leading pole to drive movement along the cell body axis and capture assembly of the motility systems
296 (FACS were not explicitly simulated on this version). Each agent is able to reverse polarity. Periodic
297 boundary conditions were applied on the simulation domain to ensure conservation of the total number
298 of agents over large simulation times.

299 We implemented the following three hypotheses: (H1) Cell reversals are triggered by frustration signal
300 resulting from deviations in individual trajectories (Fig. 4a), as suggested by our tracking data (see Fig.
301 3). (H2) As demonstrated in Guzzo et al.²⁴, and implemented in our 1D model, cells have a built-in
302 RP (T_{RP}) and a post-refractory reversal rate (T_{REV}^{-1}), both modulated by signal intensity: as frustration
303 increases, the reversal rate increases and the length of the RP decreases (Fig. 4b). (H3) Cells tend to align
304 with the ECM—a hypothesis common to both rippling and swarming—with differences in the structure
305 of the ECM in each field. In rippling fields, based on previously reported evidence in the literature^{3,38}, we
306 postulate that cells align along a given direction in areas occupied by a prey matrix (Fig. 4c), whereas in
307 swarming, we follow the hypothesis of Balagam et al. (2015), that alignment is driven by self-deposited
308 EPS only (Fig. 4d). This change in alignment rules is the only difference between rippling and swarming
309 simulations; all other mechanisms remain equal across both fields. This is supported by experimental
310 observations, which suggest that the prey matrix alters local alignment. This effect could be reinforced
311 by EPS deposition, as the cells all align over persistent distances.

312 We began with simulations of the rippling field. Following (H3), the prey matrix was mimicked
313 by forcing the cells to align over the domain along the 1D horizontal axis, reflecting the high nematic
314 order correlations observed experimentally (Fig. 1c, and fig. S2). The model was partially calibrated
315 using direct measurements (e.g. bacteria velocity, cell geometry) and the literature (maximal RP, pili
316 angular range). Beyond that, the three remaining free parameters (RP and reversal rate modulations,
317 and background alignment strength) were adjusted to obtain a bimodal TBR distribution that closely
318 matched the experimental observations (Fig. 1e).

319 We observed in our simulations that cells rapidly self-organise in counter-propagating waves that
320 resemble the rippling patterns (Fig. 5a, movie S4). We ran simulations for up to 500 minutes and
321 observed a stable dynamical pattern that never collapses. Single cell trajectories were very similar to those
322 observed experimentally (compare Figs. 5b and 1d), exhibiting quick reversals at the wave crests and

323 exhibiting a bimodal TBR (compare Figs. 5c and 1e). Noticeably, the model also captures the correlation
324 between reversals and frustrations observed in the experimental data (Fig. 5d), without additional fitting.
325 Although the mean TBR in the simulation is different from that of the experiment, we observed that the
326 simulated TBR distribution can be shifted by changing model parameters (see Supplementary Text Fig.
327 27). For example, increasing the number of cells decreases the mean TBR during rippling. Given this
328 variability, our aim was not to fit accurately the TBR distribution, but to successfully reproduce its
329 signature, i.e a bimodal profile.

330 In conclusion, our model accurately reproduces the rippling pattern and reversal dynamics observed
331 in experiments, while also replicating the signal correlation observed in the biological system.

332 We then simulated the swarming field. Again, the only change introduced from the rippling simulations
333 is the alignment rule, here restricted to interactions with self-deposited EPS trails (H3)⁸. Our simulations
334 produced patterns closely resembling the experimental swarming behavior, exhibiting the same nematic
335 order correlation over space and producing a unimodal distribution of the TBR (see Fig. 6a,b,c and movie
336 S6). Here again, we observed that the mean TBR may be shifted by varying the model parameters (for
337 example, decreasing the EPS strength increases the mean TBR in swarming, see Supplementary Text
338 Fig. 27). Since these parameters cannot be constrained by experiments, our aim was essentially to test
339 whether the experimental and simulation trends are qualitatively similar (which they are independently
340 of the variability observed in the mean TBR in our triplicate experimental data (Supplementary Text
341 Fig. 6).

342 The resulting network of streams persisted over long simulation times (up to 500 min, movie S6),
343 eventually reaching a stable configuration with only minor local rearrangements, closely matching the
344 slowly evolving networks observed experimentally (e.g., Fig. 1b and 5a,⁹). Remarkably, again without any
345 additional fitting, our simulations reproduced the observed correlation between reversals and frustration
346 (see Fig. 6d). Simulations also captured a marked difference between frustration profiles in rippling and
347 swarming fields (compare fig. S5 and Fig. 3d).

348 Finally, to control that our segmentation and tracking procedures do not skew the values of the
349 frustration index in the experimental data significantly, we tested the correlation between frustration and
350 reversals in numerical simulations of rippling and swarming (Supplementary Text, Section 5.4). With
351 perfect knowledge from the simulations, the correlation between frustration and reversals was higher than
352 that in the experimental data. We then added various levels of noise to the simulated data, and showed
353 that within the range of noise intensity that match the experimental conditions, the correlations obtained
354 match the experimental ones. Thus, our segmentation and tracking algorithms are precise enough and
355 both the TBR distributions and correlation between frustrations and reversals can be fairly interpreted.
356 Of note, the reliability of reversal detections was also confirmed by independent single-cell experiments
357 using the molecular marker SgmX-YFP, which switches polarity during reversals (see Supplementary
358 Text, Section 2)

359 After having validated our model and compared it against experimental data, we used it to disentangle
360 causal signals from correlations in reversal dynamics. Specifically, we aimed to explain why directional
361 density is seen as a potential signal during rippling but not during swarming (figs. S3 and S6a,b). This
362 could be explained for example if directional density represents an emergent byproduct of frustration-based
363 interactions due to alignment constraints in the rippling field. To test this, we first simulated rippling and

swarming with frustration-induced reversals, while recording directional density at reversal events. As expected, frustration correlated strongly with reversals in both patterns. Yet, we observed a correlation with directional density in rippling but not in swarming (fig. S6c,d). We next performed the converse simulations where directional density stands for the reversal signal then measuring frustration at reversal events. In this case, both directional density and frustration correlated with reversals in rippling and swarming, which is not observed experimentally (fig. S6e,f). We also measured the TBR distributions in these simulations, and found that only frustration reproduced the unimodal distribution of TBR observed in swarming (see fig. S7)

In conclusion, frustration-based signaling best matches experimental observations (compare fig. S6a,c,e for rippling and fig. S6b,d,f for swarming) and generate high directional density correlations in rippling fields only. This clearly suggests that such directional density is a byproduct of the strong 1D alignment and opposing movements and therefore it appears correlated, even though it may not be the driving signal.

Pattern segregation and persistence

Having established that the model reproduces each pattern separately, we next asked whether the same set of local rules—frustration-based reversals and ECM-dependent alignment—were sufficient to account for the coexistence of rippling and swarming within adjacent domains, as observed experimentally under predatory conditions (Fig. 1a,b).

To test this, we simulated a system resembling predatory conditions by defining two subdomains with the alignment rules described above: prey-induced rippling, in which cells align along some prescribed direction dictated (left side), and EPS-driven swarming, in which cells follow self-reinforced trails (right side). Cells were initially randomly distributed within each subdomain, with twice as many cells in the rippling field as in the swarming field (2:1 ratio), consistent with the experimental observation that *M. xanthus* proliferates preferentially on prey-rich areas³⁹. Under these conditions, the two patterns emerged and remained separated by a sharp, stable boundary throughout the simulation (Fig. 7a and Movie S7). Remarkably, the initial density asymmetry did not dissipate over time but remained close to a 2:1 ratio (Fig. 7b, yellow curve).

We next asked whether this density asymmetry was simply a consequence of the imposed 2:1 initial condition, or whether it reflected an emergent property of the system. To answer this, we varied the initial ratios of cells between the two fields (1:2, 1:1, 2:1, 3:1, and 4:1). In all cases, the system self-organized towards a stable density ratio favoring a higher occupancy in the rippling field (Fig. 7b). Higher density in the rippling field was observed independently of the initial ratio, even with twice more cells in the swarming area (ratio 1:2), revealing a robust emergent property of pattern coexistence. This observation raised the question of the mechanism underlying such equilibrium. Using the 2:1 initial condition as a reference, we observed that cells remain twice as long in rippling fields than in swarming fields (Fig. 7c). This increase in residence time could arise from the zigzag trajectories in rippling, where periodic wave collisions repeatedly interrupt forward motion and trap cells within the domain, suggesting that the reversal dynamics is the key mechanism behind the density asymmetry favoring the rippling field.

We therefore tested whether differences in the ability to reverse shapes cell density distributions, hence spatial patterns, in mixed populations of reversing and non-reversing cells. To do so, we simulated a mixed population consisting of reversing (Fig. 7d, red cells) and non-reversing (Fig. 7d, green cells) cells

at a 10:1 density ratio. The simulations revealed that in swarming, both cell types exhibited coordinated behavior, collectively forming cohesive swarms (Fig. 7d right). In contrast, in the rippling field (Fig. 7d left), the two sub-populations displayed distinct dynamics: reversing cells generated the characteristic colliding waves, while non-reversing cells organized into stream-like patterns instead. We next compared the residence time of non-reversing cells with that of WT reversing cells (fig. S8). Unlike the WT, non-reversing cells display a single residence time in the rippling domain, given by the domain length divided by their mean velocity (assumed to be the same for all cells in the simulation). This can be explained as all non-reversing cells simply move linearly upon entering the rippling field, being deviated only by mechanical contacts with other cells. Hence, non-reversing cells are redistributed nearly uniformly across the two areas, with no trapping effect by the rippling field, in contrast with WT cells. Thus, reversals are essential to establish and maintain the cell density ratio in favor of the rippling field. Of note, non-reversing *frzE* mutants form organized groups that move in flock-like motions but never stabilize into streams^{29,9} (compare Fig. 1b and fig. S2b). Our model could reproduce such differences when we simulated the 2D swarming behavior of the *frzE* mutant, in a single strain simulation (see Supplementary Text Fig. 28), demonstrating that it correctly captures the behavior of non-reversing cells.

To test these simulations experimentally, we mixed WT (mCherry-labelled) reversing cells and *frzE* mutant (GFP-labelled) non-reversing cells 1:10 in a predation assay. Non-reversing cells were clearly depleted from the rippling field despite the favorable initial inoculation ratio (Fig. 7e and fig. S9). We quantified the proportion of each cell type in the rippling subdomain relative to the whole population, both in simulations and experiments. In silico, this proportion was initially identical for reversing and non-reversing cells (70 % of cells in the rippling field of each cell type). Over time, simulations revealed that reversing cells remained confined within the rippling field whereas non-reversing cells became more evenly distributed between the two subdomains (Fig. 7f, left). This conclusion matched qualitatively with the strain-mixing experiments (Fig. 7f, right).

These results show that *frzE* mutant cells, which cannot reverse, fail to become trapped between waves in rippling fields, whereas the zigzag motion of WT cells facilitates their insulation within the rippling area. This differential spatial organization leads to a marked reduction of *frzE* mutant cellular density in the rippling field after 72h, as confirmed by both computational and experimental data.

Taken together these results show that cell reversals and local differences in the ECM can establish patterns that remain insulated and stable over extended periods of time. As a result, cells that do not follow these rules (here, the *frzE* mutant) are spatially sorted out of the rippling field.

Discussion

Microbial assemblies are known to form complex multicellular patterns shaped by motility, from swarms, to extreme motives such as fractal-like structures and even quasi-crystalline iridescent multicellular assemblages^{40,41,42}. It has proven extremely challenging to explain how such assemblages form from cell-cell interaction and deeper, from molecular regulations. This study shows that large-scale developmental transitions in *M. xanthus* can be explained by local spatial changes in the environment as well as the ability of the *M. xanthus* cells to resolve traffic jams by reversing. In addition, it shows that the design of the underlying regulatory system and specifically the regulation of the RP is critical to these transitions.

446 The mechanism underlying cell–cell alignment is complex. During swarming, it clearly involves in-
 447 teractions with EPS, but additional factors also contribute. Recent studies have shown that thin water
 448 layers forming on agar surfaces can promote capillary attraction between cells, which in turn favors
 449 alignment—an effect that may be modulated by surfactants³⁰. It is therefore plausible that, within the
 450 prey area, persistent alignment arises from a combination of physical and biochemical effects triggered
 451 by the presence of prey cell wall fragments³, and potentially reinforced by EPS as its deposition becomes
 452 highly aligned, ultimately resulting in the phenomenon of rippling. These fragments thus constitute an
 453 ECM capable of inducing rippling even in isolation.

454 What drives reversals? In microbes, social behaviors have long been connected to chemotaxis. Social
 455 amoeba such as *Dictyostelium discoideum*, also form cellular waves and aggregate into fruiting bodies, by
 456 using self-generated chemotactic gradients^{43,44}. In bacteria, chemotaxis drives the formation of density
 457 waves in *E. coli*^{45,46,47,48,49,50}. However, the function of chemotaxis in bacterial collectives that move on
 458 surfaces is currently unclear. While we cannot formally rule out chemotaxis in rippling or swarming, our
 459 results demonstrate that cellular alignment and the ability of cells to resolve local congestion through
 460 reversals are sufficient to explain both patterns. It is therefore more plausible that contact-dependent
 461 signals are involved rather than diffusible chemical signals.

462 Molecular components have been proposed for the mediation of contact-dependent interactions. One
 463 hypothesis suggests that C-signal, a protein expressed late in the developmental cycle, regulates reversals
 464 in a manner dependent on cell alignment (head-to-head interactions). However, the putative role of C-
 465 signal is complex, as it has been proposed both as a reversal activator during rippling and as a reversal
 466 suppressor during the subsequent aggregation stage^{51,19,52}. During rippling, C-signal is proposed to
 467 function as a surface-exposed ligand, specifically localized to the cell pole, which would activate the Frz
 468 pathway upon pole-to-pole collisions^{19,53,54,55}. This hypothesis presents several unresolved issues: (i) the
 469 polar localization of C-signal has not been experimentally demonstrated; (ii) it remains unclear how the
 470 same molecule could mediate both activation and suppression of reversals depending on the developmental
 471 stage; and (iii) the C-signal precursor protein, CsgA, is a cardiolipin phospholipase—a cytosolic enzyme
 472 involved in intracellular lipid metabolism during starvation. In fact, the enzymatic activity of CsgA
 473 explains most of the C-signal mutant phenotypes²¹.

474 Alternatively, we propose that reversals may be triggered by mechanical cues. It seems difficult to
 475 provide a direct demonstration of this mechanism, and we rather rely on the frustration indexes which
 476 are indirect proxies. In congested regions, mechanical stress does not arise solely from head-on collisions
 477 because the cells frequently slide past one another laterally. Instead, stress likely accumulates through
 478 the multitude of interactions that occur as cells move into crowded areas. Recent studies^{56,57} show that
 479 congestion sites, identified by $+1/2$ topological defects in the nematic field, are indeed associated with
 480 strong fluctuations in the traction force. As in any traffic jam, these effects are likely to propagate over
 481 long distances. We propose that such potential stress can be relieved through reversals. Mechanosensing
 482 could be mediated by the motility motors themselves—for instance, as load accumulates at the sites of
 483 congestion. A similar mechanism is proposed in *Pseudomonas*, where Type-IV pili—the same motility
 484 apparatus found in *M. xanthus*—activates a reversal control system (Chp, a chemosensory-like pathway)
 485 upon cell-cell collisions^{58,59}. We hypothesize that *M. xanthus* cells monitor their local environment by
 486 integrating mechanical signals as they navigate congested regions, eventually reaching a threshold that

487 triggers a reversal. Mechanisms such as receptor adaptation, similar to those described in chemotaxis—
488 e.g., through methylation and demethylation of FrzCD—may mediate this regulation. Indeed, various
489 levels of sensitivity could explain the observed cell-cell variability in responding to frustration levels.

490 At the molecular level, regulation of the RP²⁴ explains the measured TBRs both in swarming and
491 rippling fields because it enables short reversals in congested areas. In rippling fields, where the cells
492 are highly aligned, this leads to synchronisation at the population level. In the swarming field, where
493 the cells are constrained in narrow EPS trails, short reversals dominate because of numerous congestion
494 and likely this participates in their alleviation. The importance of RP modulation is emphasized by
495 the analysis of updated mathematical models^{12,15}. Rippling formation is largely facilitated when RP is
496 regulated as compared with constant RP. The former allows sensitivity across a much wider range of
497 signalling values thereby reducing the stringency on the nonlinear feedback needed for the emergence of
498 collective dynamics. A direct parallel can be made with the RP regulation favouring the synchronisation
499 of electrical discharges in networks of excitatory neurons, via so-called synaptic inhibition^{60,61}.

500 In mammals, tissue formation often results from spatial sorting where cell migration to specific sites
501 precedes the onset of a genetic program leading to tissue differentiation. Our study sheds a new light on the
502 spatial organization of bacteria preceding fruiting body formation in the *M. xanthus* cycle, where swarming
503 and rippling patterns remain adjacent over extended periods. Cells transitioning from swarming to rippling
504 fields integrate into rippling waves within minutes—too rapidly to depend on de novo synthesis of rippling-
505 specific determinants such as the putative C-signal. Our model indicates that immediate transcriptional
506 reprogramming is not required for these two domains to form and coexist. The two spatial domains
507 are physiologically distinct: rippling follows predation, whereas swarming occurs under nutrient-depleted
508 conditions. Consistently, fruiting body formation is delayed in areas where prey is present⁶². Thus, while
509 transcriptional regulation may not play an immediate role in the swarming-to-rippling transition, it is
510 likely that distinct gene expression programs are subsequently activated within these adjacent regions.

511 In addition to forming a stable spatial domain, rippling may serve to insulate cells that have gone
512 through the predation cycle or to eliminate mutants with impaired motility. Since rippling precedes
513 fruiting body formation, it could function as a selective sieve, determining which cells will ultimately
514 become encapsulated as spores. We present a proof of principle demonstrating that, for instance, *frzE*
515 mutant cells are gradually expelled from the rippling field and are therefore unlikely to be incorporated
516 into the final spore population. Although rippling is stable over time, it eventually dissipates to allow
517 aggregation to proceed. How does this transition occur? Rippling collapses as cell density decreases,
518 a condition that may arise due to programmed cell death occurring late in starvation⁶³. Additionally,
519 cells may shift their developmental program by suppressing reversal behavior as starvation progresses⁶⁴.
520 Indeed, such a change could simply explain the shift to fruiting body formation, with multilayers emerging
521 at congestion sites due to mechanical stress⁵⁷.

522 In bacteria, such spatial differentiation mechanisms are likely pervasive as well. One example is spatial
523 sorting, *i.e.* the selection of phenotypes through space rather than time⁶⁵, which has been demonstrated in
524 both experimental and theoretical studies of microbial populations. Differences in motility or chemotactic
525 responsiveness can drive spatial differentiation, allowing for stable coexistence or exclusion even in the
526 absence of genetic variation^{66,67}. This has been shown experimentally in *E. coli* populations and supported
527 by mathematical models of bacterial systems^{50,68}, exhibiting spatial self-organization during collective

528 migration. These studies support the idea that motility differences alone can lead to robust spatial
 529 separation in structured bacterial communities, even in the absence of genetic differences. Future work
 530 will test whether the principles identified here apply to other systems—both bacterial and beyond.

531 In flagellated bacteria, for instance, the single-cell rules governing swarming remain to be fully de-
 532 fined, although tracking experiments have shown that tumbling is suppressed in active swarms⁶⁹. More
 533 broadly, this study highlights the central role of individual alignment in the emergence of collective or-
 534 der, echoing the mechanisms underlying schooling in fish, where visual and hydrodynamic cues maintain
 535 short-range alignment and coordinated motion^{70,71,72}. Ultimately, such approaches may help establish
 536 unifying frameworks for understanding collective behaviors across scales, from bacterial communities to
 537 human crowds

538 Finally, while kinetic equations were originally developed for molecular gas dynamics⁷³, they prove re-
 539 markably effective for analyzing the cellular and molecular basis of multicellular patterning when extended
 540 with biological components, sensory cues, and regulatory networks governing cellular decisions. The anal-
 541 ysis of the one-dimensional kinetic model, and in particular the existence of spatio-temporal, diamond-like,
 542 periodic patterns (Fig. 2c) remains an open problem (but see¹⁵). Combined with high-resolution time-
 543 lapse microscopy, these models provide a foundation for deriving mesoscopic and macroscopic descriptions
 544 grounded in microscopic rules. Bridging these scales for contact-based interactions remains a major the-
 545 oretical challenge, requiring approaches beyond standard mean-field models, yet the wealth of single-cell
 546 data now available offers promising avenues for developing quantitative, multiscale frameworks.

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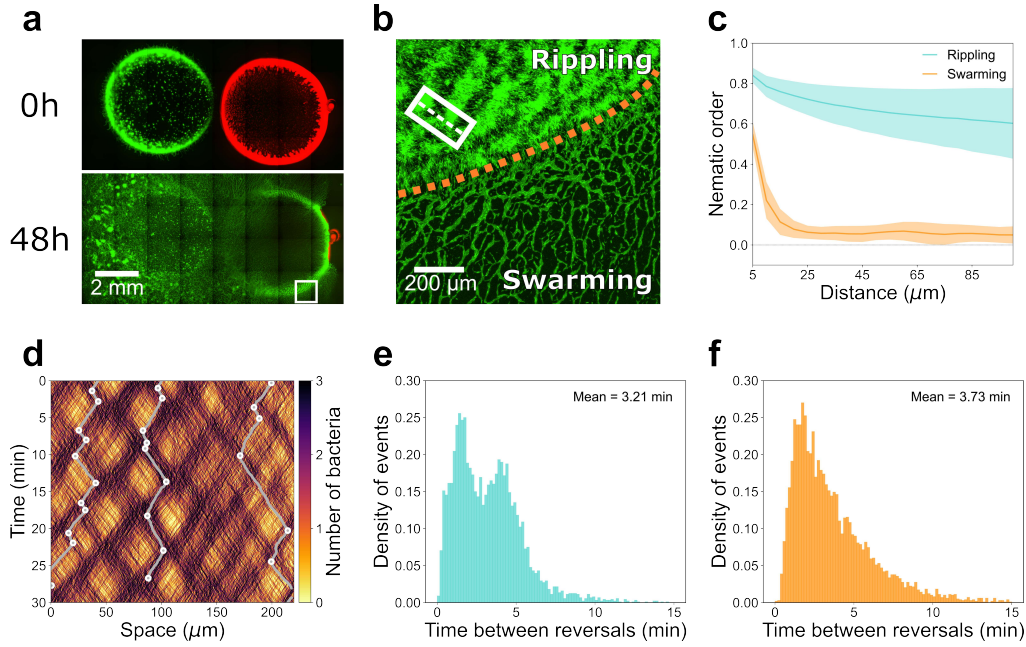


Figure 1: Single cell behaviors during *M. xanthus* swarming and rippling. (a) Predation assay of *M. xanthus* (green, GFP) against the prey *E. coli* (red, mCherry). When a *M. xanthus* colony is plated next to an *E. coli* colony it invades and digests it within 48 h. (b) Rippling and swarming coexist around the prey colony boundary. Shown is a zoom from panel (a), corresponding to the white square, showing the formation of rippling waves that form inside the prey colony, once the prey has been entirely digested. Swarming behaviors are observed in the prey-free areas. The interface between rippling and swarming is shown in an orange dashed line. (c) Cell alignment in rippling and swarming areas. Shown is the mean nematic order of bacteria (rod-shaped cells) as a function of the distance. Blue and orange indicate either the rippling field, or the swarming field, respectively. (d) Kymograph of single cell trajectories observed in rippling fields. The kymograph corresponds to the section delineated in the white rectangle shown in (b). As examples, three individual cell tracks are shown in grey, and the white circles indicate the reversal events. (e, f) Single-cell reversals in rippling and swarming areas. Shown are the distributions of the time between reversals (TBR) for single cells in rippling (e) ($N = 5283$ TBR events) and swarming areas (f) ($N = 5904$ TBR events) in one representative experiment. Only trajectories longer than 1000 frames (33 min) were included to reduce bias from short-lived tracks. Note that although the mean TBR varies between experiments (see Supplementary Text Fig. 6), all replicates show unimodal distributions for swarming and bimodal distributions for rippling.

a**LOW SIGNAL:**

$$\begin{aligned}
& \underbrace{u_t^\pm(t, x, r)}_{\text{Time evolution}} \pm \underbrace{v u_x^\pm(t, x, r)}_{\text{Right-left movement}} + \underbrace{u_r^\pm(t, x, r)}_{\text{Time count since last reversal}} = \underbrace{-T_{REV}^{-1}(\rho^\mp) u^\pm(t, x, r) \mathbf{H}(r - R^*)}_{\text{Reversal events}} \\
& u^\pm(t, x, r = 0) = T_{REV}^{-1}(\rho^\pm) \int_0^{+\infty} u^\mp(t, x, r) \mathbf{H}(r - R^*) dr
\end{aligned}$$

HIGH SIGNAL:

$$\begin{aligned}
& u_t^\pm(t, x, r) \pm v u_x^\pm(t, x, r) + u_r^\pm(t, x, r) = -F^* u^\pm(t, x, r) \mathbf{H}(r - T_{RP}(\rho^\mp)) \\
& u^\pm(t, x, r = 0) = F^* \int_0^{+\infty} u^\mp(t, x, r) \mathbf{H}(r - T_{RP}(\rho^\pm)) dr
\end{aligned}$$

DIRECTIONAL DENSITY SIGNAL:

$$\rho^\pm(t, x) = \int_0^{+\infty} u^\pm(t, x, r) dr$$

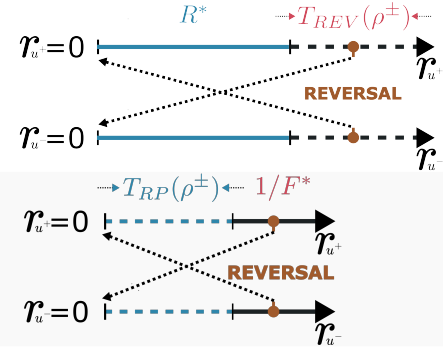
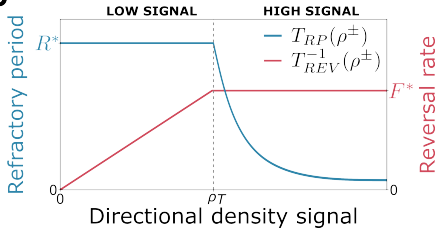
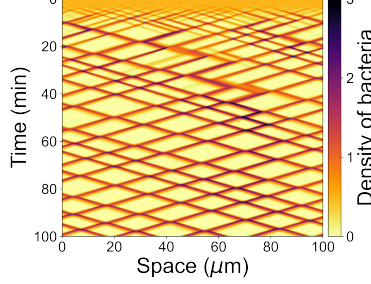
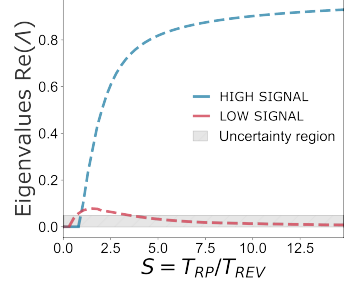
**b****c****d**

Figure 2: Regulation of the refractory period promotes multicellular pattern formation. (a) Equations governing the 1D age-structured model. The functions $u^+(t, x, r)$ and $u^-(t, x, r)$ represent the densities of cells moving to the right and left, respectively, at time t , position x , and age r , defined as the time since the last reversal. Cells move at constant speed v . The model includes two time scales: T_{RP} and T_{REV} . Cells cannot reverse within the refractory period ($r < T_{RP}$). Beyond the refractory period, the rate of reversal per unit of time is T_{REV}^{-1} (mean time T_{REV}). The Heaviside function $\mathbf{H}$ in the PDE ensures that reversals occur provided that $r > T_{RP}$. Upon reversal, the variable r is reset to zero, and the cell switches direction. The dynamics depend on the signal, defined as the density of opposite cells $\rho^\pm(t, x)$, that is, either $\rho^+(t, x)$ or $\rho^-(t, x)$. Two regimes are considered: Low signal: the refractory period is fixed at its maximum value R^* , while the reversal rate T_{REV}^{-1} increases with $\rho^\pm$, leading to faster reversal for a larger signal. High signal: the reversal rate saturates at its maximum value F^* , while the refractory period T_{RP} shortens as $\rho^\pm$ increases. The right-hand diagrams illustrate the reversal mechanism in both regimes: cells become eligible for reversal once their age exceeds the refractory period (blue bar), and can then reverse with a rate which is either signal-dependent or constant (maximal). (b) Refractory period $T_{RP}(\rho)$ (solid curve) and rate of reversal $T_{REV}^{-1}(\rho)$ (dashdotted curve) as functions of the signal. The density ρ_T is the threshold separating low and high signaling regimes. In the low signaling regime, the refractory period T_{RP} is constant and equal to R^* , whereas the reversal rate is modulated and increases linearly with the signal: $T_{REV}^{-1}(\rho) = \frac{F^*}{\rho_T} \rho$. In the high signaling regime, the reversal rate reaches its maximum $T_{REV}^{-1} = F^*$, whereas the refractory period is modulated and decreases with the signal as $T_{RP}(\rho) = \frac{R^* \rho_T}{\rho}$. (c) The age-structured model produces rippling. Kymograph of the one-dimensional simulation of the age-structured model with directional density signal (the same result is obtained with a local density signal, see Supplementary Text Fig. 10a), illustrating the emergence of counter-propagating waves, stabilizing in a periodic spatio-temporal pattern. (d) Modulation of the refractory period facilitates pattern formation. Instability analysis of the homogeneous steady state, showing the dominant eigenvalue Λ as a function of the ratio $S = T_{RP}(\bar{\rho})/T_{REV}(\bar{\rho})$, where $\bar{\rho}$ is the steady-state density. In the high signal regime (blue curve), where the refractory period is modulated, Λ becomes positive beyond a threshold, indicating the emergence of an unstable mode and the onset of pattern formation. In contrast, in the low signal regime (red curve), where only the reversal rate is modulated, Λ remains close to zero, within the range of numerical errors (grey zone) and instability can only arise at a very low pace (no instability is observed in the case of local density signaling, see Supplementary Text Fig. 13).

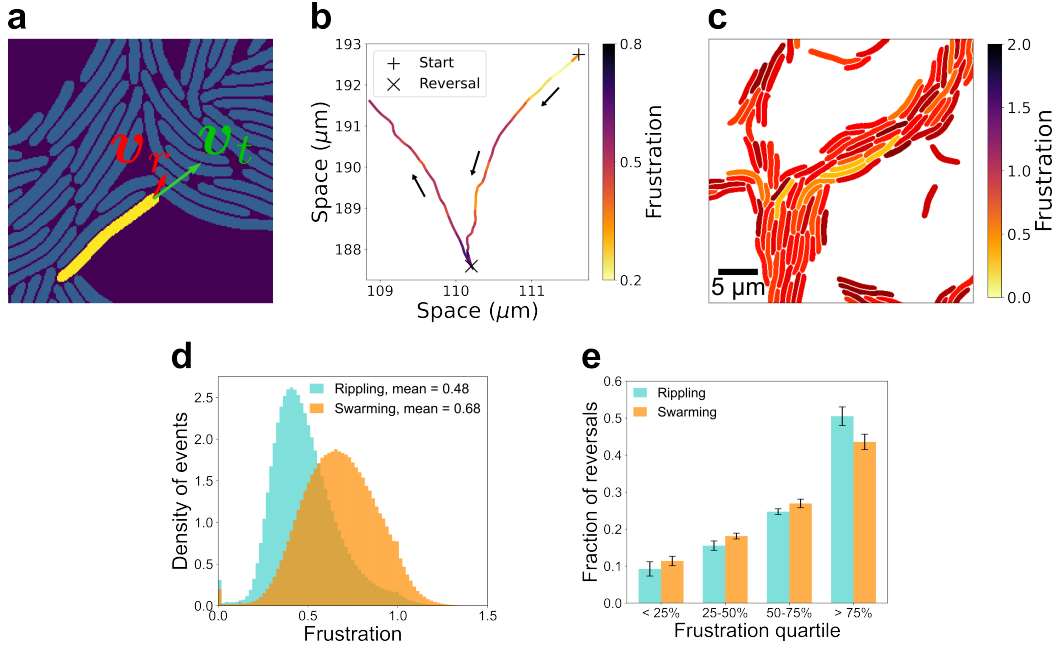


Figure 3: *M. xanthus* reversals are activated in cellular congestions. (a) Frustrations in bacterial cell groups. Here, $\mathbf{v}_t$ is the target velocity (i.e. the velocity that would likely have been observed had the bacterium continued moving in the absence of an obstacle) and $\mathbf{v}_r$ is the observed velocity (which is measured experimentally). A visual example is provided with the cell labelled in yellow (see movie S8). Our frustration index is given by $f = 1 - \frac{\mathbf{v}_t \cdot \mathbf{v}_r}{\max(\|\mathbf{v}_r\|^2, \|\mathbf{v}_t\|^2)}$. It is computed for each cell at each time step along its trajectory, then averaged over 9 time frames to smoothen the signal. (b) Trajectory of a single *M. xanthus* cell and its associated frustration levels. A reversal event is marked with a cross (x). Note the accumulation of frustration prior to the reversal event. (c) Mapping frustration levels in swarms. Each bacterium is shown color coded with its frustration level. (d) Frustration distributions in rippling and swarming, computed over 3 independent experiments (movies) with $N = 13.7 \times 10^6$ frustration events in rippling and $N = 12.8 \times 10^6$ in swarming. Each movie consists of 1800 frames captured every 2s. (e) Frustrated cells have a high propensity to reverse. For each individual cell trajectory, the frustration value was recorded at every time frame, yielding a trajectory-specific distribution of frustration values. For each trajectory, this distribution was then divided into four quartiles: relatively low frustration (below the 25 % percentile), intermediate low (25 % to 50 %), intermediate high (50 % to 75 %), and relatively high frustration (above the 75 % percentile). Bar plots show the proportion of reversal events occurring when cells are in each of these trajectory-specific frustration quartiles (see Supplementary Text, Section 5, and Fig. S4 for details). For instance, in the rippling area (blue), more than 50 % of reversals occur when the instantaneous frustration belongs to the highest quartile of the cell’s own frustration history. Mean $\pm$ SD values of reversal fractions for rippling cells are 9.2 ± 1.9 %, 15.5 ± 1.3 %, 24.7 ± 0.8 %, and 50.5 ± 2.5 % from the first to the fourth quartile, respectively; for swarming cells they are 11.4 ± 1.3 %, 18.1 ± 0.8 %, 26.9 ± 1.2 %, and 43.6 ± 2.1 %. Analysis was conducted on rippling cells (cyan, $N = 222\,769$ reversals analyzed) and swarming cells (orange, $N = 70\,406$ reversals analyzed). Error bars represent the standard deviation.

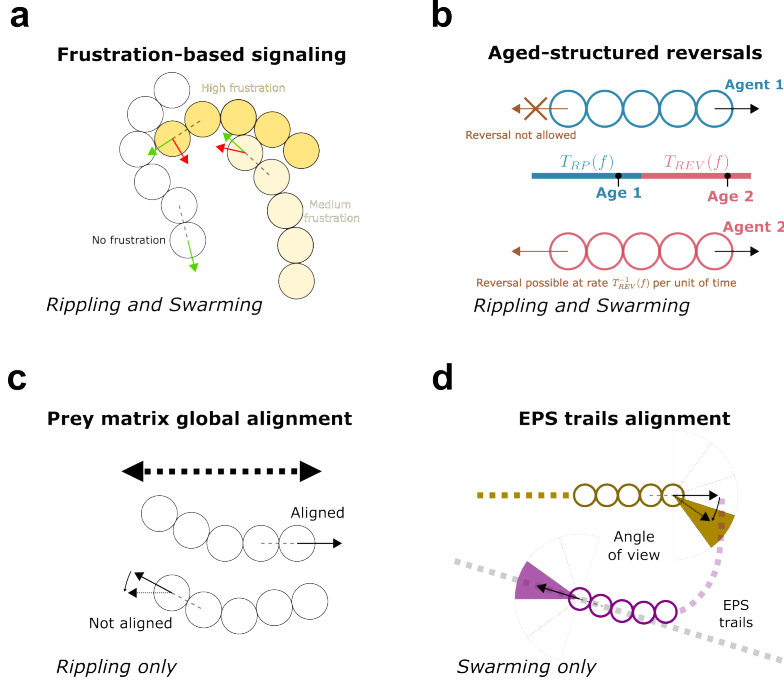


Figure 4: Hypotheses for *M. xanthus* behaviour in the 2D agent-based model. (a) Frustration-based signaling. In this framework, the probability of reversal increases with frustration (f), defined as the deviation (red arrows) from the intended trajectory (green arrows). (b) Age-structured reversals. After each reversal, a refractory period ($T_{RP}(f)$) prevents new reversals (Agent 1). Once this period has elapsed, the agent enters a sensitive phase and reversals occur at a rate $T_{REV}^{-1}(f)$ (Agent 2). The explicit functions for $T_{RP}(f)$ and $T_{REV}^{-1}(f)$ are smooth versions of Fig. 2b provided in Supplementary Text Fig. 26. (c) Global alignment in rippling. In rippling regions, the prey matrix promotes global alignment, leading agents to orient collectively in one direction, prescribed by the prey matrix's external field. (d) Trail-mediated alignment in swarming. In swarming regions, alignment is guided by EPS trails. Moving agents deposit trails that can be detected by others within their field of view, which then adjust their orientation to follow them.

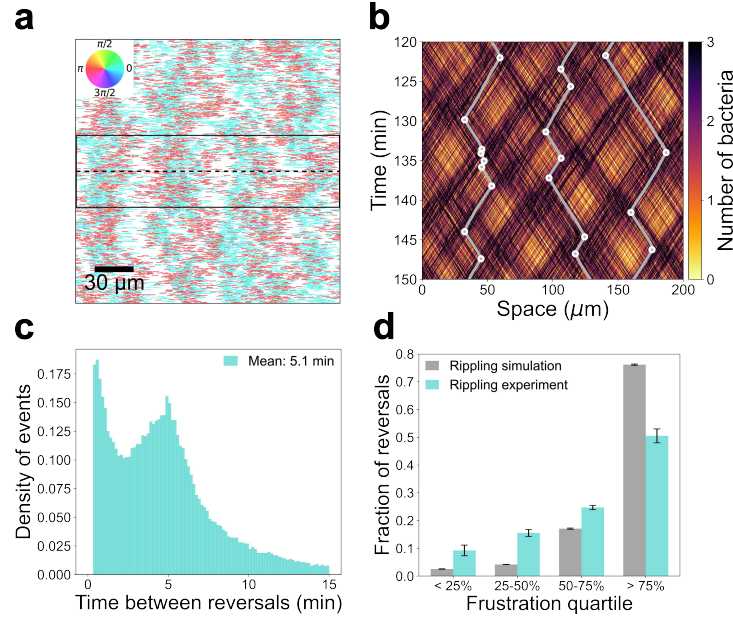


Figure 5: A 2D agent-based model captures the essential features of rippling. (a) Rippling patterns are observed in the simulation. The pattern corresponds to $N = 9000$ cells after 500 min. See corresponding movie S4. (b) Kymograph of single cell trajectories across the boxed section (dotted line) shown in (a). Three tracks are added in white, the circles indicate reversal events. (c) Rippling simulations reproduce the distribution of time between reversals observed experimentally. Shown is the distribution of time intervals between successive reversals obtained from simulations ($N = 88679$ time-between-reversal intervals). (d) Rippling simulations reproduce the dependence of reversal events on frustration. Shown is the distribution of reversal events across frustration quartiles in simulations (grey, $N = 331640$ reversals analyzed) and experiments (cyan, see Fig. 3e). Mean reversal fractions in simulations are 2.6 %, 4.2 %, 17.1 %, and 76.2 % from the first to the fourth quartile, respectively. For rippling simulations, standard deviations are negligible.

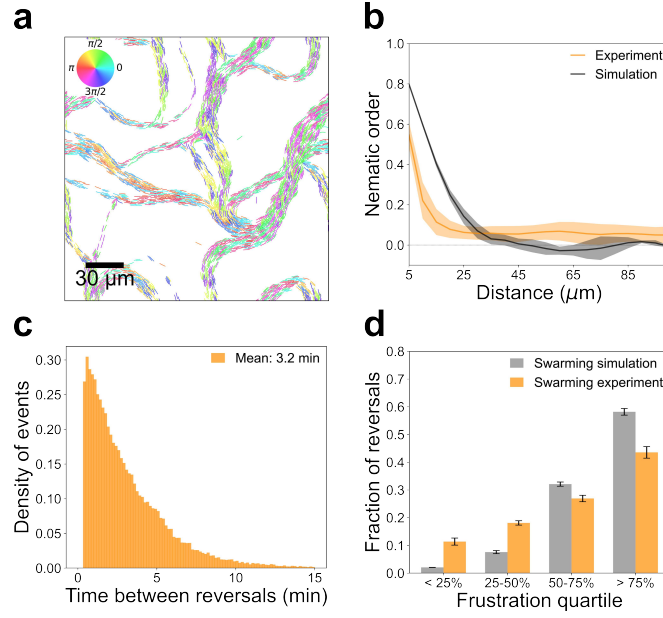


Figure 6: The same 2D agent-based model captures the essential features of swarming. (a) Swarming patterns are observed in the simulation. The pattern corresponds to $N = 3330$ cells after 500 min. See corresponding movie S6. The difference with the simulations of Fig. 5 lie in the interactions with the ECM (here, alignment along self-deposited EPS). (b-d) The simulation reproduces single cell population features as observed in the experiments. (b) Alignment: cell alignment in simulations shown by the mean nematic order as a function of the distance and comparison with experiments. (c) Distribution of the time between reversals captured by the simulation ($N = 50\,927$ TBR intervals). (d) Distribution of reversal events according to their level of frustration in simulations (grey, $N = 191\,535$ reversals analyzed) and in experiments (orange, see Fig. 3e). Mean $\pm$ SD values of reversal fractions in simulations are $2.1 \pm 0.03\%$, $7.6 \pm 0.6\%$, $32.1 \pm 0.8\%$, and $58.2 \pm 1.2\%$ from the first to the fourth quartile, respectively.

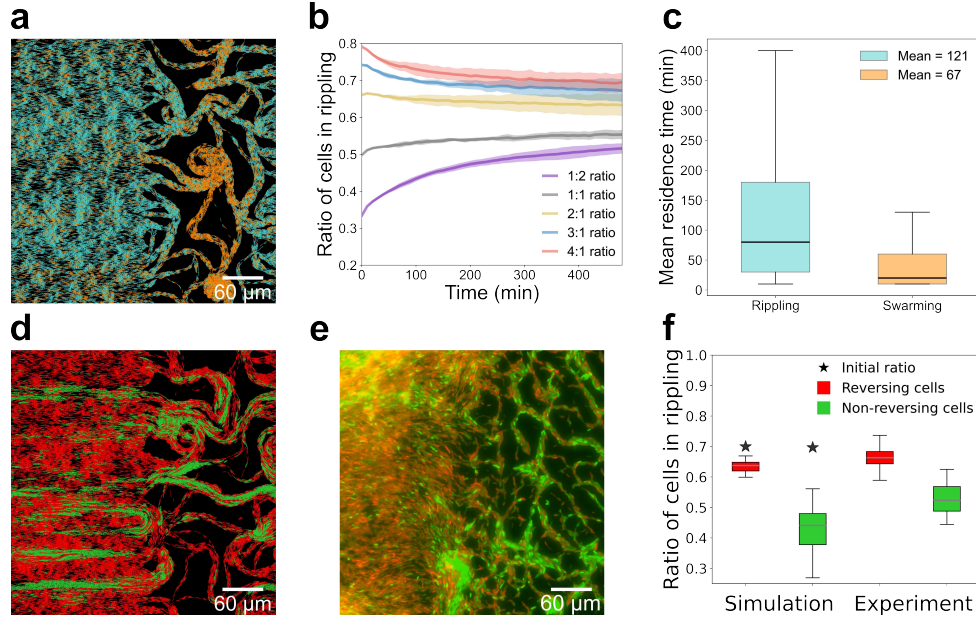


Figure 7: The simulations explain the stable co-existence of rippling and swarming fields over long periods of time. (a) Simulations capture the rippling-swarming transition. Shown is the result after a 500 min simulation with reversing agents. The cells were forced either to align along the horizontal direction on the left side, mimicking the prey matrix, or to follow self-deposited EPS trails on the right side. See corresponding movie S7. (b) Cell densities are stable in rippling and swarming fields. Shown is the time evolution of the ratio of cells in the rippling area over the total number of cells ($\frac{N_{\text{rippling}}}{N_{\text{total}}}$) along the entire simulation of panel (a) for five distinct initial ratios. (c) Rippling dynamics trap the cells. Shown are the mean residence times of cells in rippling versus swarming domains after 500 min of simulation. For each cell, continuous episodes of presence within one domain (from entry until exit) were identified starting from $t_0 = 100$ min, when the patterns were fully settled, until the final time $T = 500$ min. The duration of these episodes was averaged across all visits and across five independent simulations with the 2:1 ratio as initial condition. Boxplots show the distribution of mean residence times per simulation, highlighting that cells remain significantly longer in rippling fields than in swarming fields. (d) Reversal ability shapes spatial organization in rippling and swarming fields. Shown is a simulation of a mixed population of reversing (red) and non-reversing (green) cells at a 10:1 density ratio after 500 min of simulation time. In the swarming field (right), both cell types exhibit coordinated collective motion and form cohesive swarms. In contrast, in the rippling field (left), the two sub-populations display distinct dynamics: reversing cells generate the characteristic colliding waves, while non-reversing cells organize into stream-like patterns. See corresponding Movie S7. (e) Experiment corresponding to the simulation shown in (d). The rippling-swarming transition is shown after mixing reversing (red, mCherry) and non-reversing (green, GFP) bacteria in a predatory assay. (f) Non-reversing cells do not persist in rippling fields in simulations and experiments. In the simulation (d), the same ratio as in panel (b) ($\frac{N_{\text{rippling}}}{N_{\text{total}}}$) was quantified after 100 min, long after the rippling and swarming patterns are settled. The initial ratio of cells in the simulation is represented by a dark star. For the experimental data, several ratios were computed from a selection of different subareas ($n = 22$) in the entire field of experiment (fig. S9) around the border of the prey area, such as the subarea shown in panel (e). Each ratio (reversing or non-reversing) was computed within its own color channel (red or green).

707 Methods

708 Bacterial strains and growth conditions

709 Strains used for this study are listed in table S1. *M. xanthus* strains were grown at 32 °C in CYE (Casitone
710 Yeast Extract) rich media as previously described⁷⁴.

712 Microscopy

713 To image the rippling and swarming patterns of *M. xanthus* in a predatory assay, both *M. xanthus* and
714 *E. coli* cells were grown in CYE, pelleted, and resuspended in CF medium to a final OD₆₀₀ of 5. Equal
715 volumes (1 µL) of the two suspensions were then spotted side by side on a freshly prepared CF 1.5 % agar
716 pad on a microscope slide. After the spot has dried, the agar pad was covered with a glass coverslip,
717 and incubated in the dark at room temperature for 48 h before imaging. Time-lapse microscopy was
718 performed using an inverted epifluorescence Nikon Eclipse Ti2 microscope, equipped with a 100×/1.4 NA
719 DLL oil-immersion objective and a Teledyne Photometrics Kinetix camera. This microscope also features
720 a “Perfect Focus System” (PFS), which automatically maintains focus, even in the presence of mechanical
721 or thermal perturbations. Phase-contrast movies were recorded for 1 h, with images acquired every 2 s.
722 In selected experiments, fluorescence excitation was provided by a LED illumination system with YFP
723 filter cubes (508 nm excitation, 15 % power), and fluorescence images were acquired every 10 s with 100 ms
724 exposure time. Images were acquired using NIS-Elements (Nikon).

726 Cell tracking

727 The phase contrast movies were segmented with Omnipose⁷⁵ and tracked using a custom tracking al-
728 gorithm. See Supplementary Text, Section 1 "Segmentation and tracking" for detailed description and
729 justification.

731 Radial nematic order correlation function

732 We define a ring as the region between two concentric circles of radius $r - \epsilon/2$ and $r + \epsilon/2$ (for a fixed ϵ),
733 within which we compute the nematic order for each radius r , with r ranging from 5 µm to 100 µm. We
734 denote the nematic order as $\mathcal{O}(r)$ and use the following expression²⁷:

$$\mathcal{O}(r) = \left\langle \frac{1}{N_i(r)} \sum_{j=1}^{N_i(r)} [2 \cos^2(\theta_i - \theta_j(r)) - 1] \right\rangle_i,$$

735 where θ_i is the orientation of bacterium i , $N_i(r)$ is its number of neighbours within the ring, and $\theta_j(r)$
736 is the orientation of its j^{th} neighbour. The maximal radius (100 µm) corresponds to half the size of the
737 microscopic field of view. Standard deviations were computed from triplicates for both experiments and
738 simulations. The orientation of the bacteria was extracted from phase-contrast images using the His-
739 togram of Oriented Gradients (HOG) method from the `skimage.feature` Python library^{76,77}.

741 Reversal detection

742 The detection of reversals was performed by identifying abrupt changes in the direction of cell trajec-
743 tories (e.g., acute angles). These trajectories are smoothed beforehand to filter out artificial directional

changes. This method was meticulously compared to a molecular-based detection method, based on the pole relocalization of the SgmX protein, and was shown to be robust and consistent with this molecular assay. See Supplementary Text, Section 2 "Reversals detection" for detailed description and justification.

Kymographs

Kymographs are generated from both rippling movies and simulations by projecting cell positions along an axis defined by the mean direction of the cells across the entire time lapse. For each frame of the movie, the resulting projections were arranged sequentially in temporal order, forming a stacked representation. Colors in the kymographs indicate the number of cells projected at each position.

The 1D model and the stability analysis

Building upon the work Degond et al.¹⁴, we designed at first the age-structured model, which is a Partial Differential Equation (PDE) of kinetic-transport type. It describes the evolution of *M. xanthus* cell density in time, space (1D) and a third variable recording the time since last reversal. The latter variable is essential to investigate the role of the RP. The phase-structured model is another PDE of kinetic-transport type, used here to test the robustness of our conclusions. Based on a series of works^{11,12}, it differs from the previous one by the nature of the variable encoding the period between two reversals. Instead of a time, it is now a phase which should make it fully across a fixed interval $[0, \pi]$ for a reversal to occur. Kinetic models are well-suited for describing the persistent motion of cells, and proved adequate to capture chemotactic waves of *E. coli* in microchannels by an accurate description of the run-and-tumble motion⁴⁸. Moreover, in contrast with agent-based models, PDE models are suitable for pattern formation analysis. For the stability analysis, we performed linear stability analysis in Fourier modes with respect to the spatial variable, by introducing fluctuations around the homogeneous steady state. We then numerically computed the eigenvalues (the growth rate Λ) of the matrix operator driving the fluctuations with a Python code. The singular terms should be handled with care, in particular for the linearization of the model with modulation of the RP, which naturally introduces Dirac masses in the analysis. See the main text and Supplementary Text, Section 4 "A pair of mathematical models for the emergence of the rippling collective stage" for detailed description and justification.

Signal metrics

To characterize the local signals potentially triggering reversal events, we define three quantities at the single-cell level: frustration, local density, and directional density.

The *frustration* (denoted $f^i(t)$) of each bacterium i at each time step t is computed as the deviation between the bacterium's target velocity $\mathbf{v}_t^i(t)$, defined as the velocity aligned with the cell body with constant speed $4 \mu\text{m min}^{-1}$, and the real cell velocity $\mathbf{v}_r^i(t)$ obtained from its head displacement between two consecutive frames. The frustration formula is given by:

$$f^i(t) = 1 - \frac{\mathbf{v}_t^i(t) \cdot \mathbf{v}_r^i(t)}{\max(\|\mathbf{v}_t^i(t)\|^2, \|\mathbf{v}_r^i(t)\|^2)}.$$

See the main text and Supplementary Text, Section 5 "Frustration definition and related figures" for a detailed description and justification.

782 *The local density* is defined as the number of neighboring bacteria in direct physical contact with bac-
783 terium i . Each bacterium is represented by eleven equidistant points sampled along its skeleton, which
784 is extracted using the `skan` Python library. Two bacteria are considered to be in contact if the minimal
785 Euclidean distance between any skeleton point of bacterium i and any skeleton point of bacterium j is
786 strictly smaller than $0.84\ \mu\text{m}$. This threshold is chosen based on the experimentally measured average cell
787 width of $0.7\ \mu\text{m}$.

788 *The directional density* quantifies the number of neighboring bacteria in physical contact that move in
789 an opposing direction relative to bacterium i . Only neighbors defined by the local density criterion are
790 considered. For each bacterium, a mean orientation angle is computed from its skeleton using the circular
791 mean of the skeleton segment orientations. A neighboring bacterium is considered to move in an opposing
792 direction if the scalar product between the unit velocity vectors corresponding to the mean orientations
793 of the two bacteria is strictly negative.

794 795 **The 2D model**

796 Building on the work of Balagam and Igoshin⁸, we developed a 2D model for the collective movement of
797 *M. xanthus*⁷⁸. In this model, each cell is represented as a series of spheres (10 in this case) connected by
798 springs, with a fixed distance between them. The movement of the head sphere drives the movement of
799 the other spheres, giving each cell its motion. Overlap between spheres was prevented by introducing a
800 distance-dependent repulsion force between sphere centres. The reversal mechanism mirrors that of the
801 1D model, with the frustration signal replacing the local and directional density signals. In the rippling
802 field, agents align in the horizontal direction, while during swarming, they follow EPS-trails generated by
803 the cell themselves. The cells are placed in a square domain with periodic boundary conditions. See the
804 main text and Supplementary Text, Section 6 "Modeling of the 2D simulations" for detailed description
805 and justification.

806 807 **Residence time in rippling and swarming domains (Fig. 7c)**

808 From $t_0 = 20\ \text{min}$ (when the patterns are established), we computed, for each cell, the residence time per
809 visit in the rippling and swarming domains. A visit is defined as a contiguous sequence of frames during
810 which the domain label remains unchanged, with its duration (in minutes) given by the number of frames
811 in the sequence multiplied by the frame period. Residence times are conditioned on occupancy, meaning
812 that only cells that visited a given domain at least once contribute to its distribution (zero-residence
813 events are excluded). The figure shows boxplots (outliers hidden) of the per-visit residence times pooled
814 across triplicate simulations for each domain ("Rippling", "Swarming"); the median is indicated by the
815 central line, and the legend reports the mean of each distribution.

816 817 **Ratio of reversing versus non-reversing cells in rippling (Fig. 7f)**

818 We define $N_R^{\text{rev}}(t_i)$ and $N_R^{\overline{\text{rev}}}(t_i)$, for $i \in [t_1, \dots, t_f]$ with $t_1 = 100\ \text{min}$ and $t_f = 250\ \text{min}$, as the number
819 of reversing and non-reversing bacteria located in the rippling field at time t_i . We also define N^{rev} and
820 $N^{\overline{\text{rev}}}$ as the total numbers of reversing and non-reversing bacteria in the simulation. We then computed
821 the ratios $N_R^{\text{rev}}(t_i)/N^{\text{rev}}$ (red boxplots) and $N_R^{\overline{\text{rev}}}(t_i)/N^{\overline{\text{rev}}}$ (green boxplots). For the experimental data,
822 the same ratios (defined above) were calculated over 22 fields located at the edge of the prey area (post-

823 predation). The stars indicate the ratios $N_R^{\text{rev}}(t=0)/N^{\text{rev}}$ and $N_R^{\overline{\text{rev}}}(t=0)/N^{\overline{\text{rev}}}$.
824

825 **Fitting procedure of the 2D model**

826 The parameters of the 2D model are divided into four categories based on their origin and role in the
827 simulation.

828 1. Parameters from the literature

829 These parameters are derived directly from previous studies and provide biologically accurate baseline
830 values:

- 831 - Maximal refractory period (R^*): Set to 5 min, as reported in Guzzo et al.²⁴.
- 832 - Distance between consecutive disks (ε) and number of disks per bacterium (N_D): Set to 0.5 μm and 10,
833 respectively, corresponding to a total bacterial length of 5 μm , which matches average values reported in
834 the literature⁷⁹.

- 835 - Horizon search (H): Set to approximately one cell length (about 5 μm)⁶.

- 836 - Minimal detectable EPS concentration ($c_{\min}$): Set to 0.1, based on Balagam and Igoshin⁸.

837 2. Parameters extracted from experimental measurements:

- 838 - Diameter of disks (D): Set to 0.7 μm , matching the experimentally measured bacterial width.

- 839 - Bacterial velocity (v): Set to the average speed observed experimentally, 4 $\mu\text{m min}^{-1}$.

- 840 - Minimal refractory period ($R_{\min}$): Set to 20 s, extracted from the distribution of times between reversals.

841 3. Ad hoc parameters

842 These parameters were chosen to ensure that the model obeys physical laws and behaves realistically:

- 843 - Hooke's spring constant (k_s): Set to 50 min^{-1} to maintain the spacing between disks and preserve cell-
844 body structure.

- 845 - Repulsion coefficient (k_r): Set to 450 $\mu\text{m}^{-1} \text{min}^{-1}$ to prevent overlap between simulated bacteria.

- 846 - Angle of view (2α): Set to π , modelling the pilus exploration area as a semicircle in front of the cell
847 head.

- 848 - EPS deposition rate (a) and maximum EPS grid value ($\text{EPS}_{\max}$): Set to 2 min^{-1} and 10, respectively,
849 to ensure sufficiently rapid EPS trail formation.

- 850 - Rippling alignment strength (γ'): Set to 11 $\mu\text{m min}^{-1}$ to ensure 1D alignment.

- 851 - EPS alignment strength (γ): Set to 11 $\mu\text{m min}^{-1}$ to ensure 2D alignment with EPS trails.

- 852 - Maximum reversal rate (F^*): Set to 30 min^{-1} to obtain a realistic number of reversals.

- 853 - Frustration memory (d) and memory kernel exponent (β): Set to 20 s and 2 min, respectively, following
854 chemotaxis-inspired models⁸⁰, giving more weight to the recent past.

855 4. Unknown parameters

856 These parameters were adjusted to match experimentally observed TBR distributions:

- 857 - Frustration threshold (f_T): Set to 0.08.

- 858 - Steepness of the reversal rate function (α_F): Set to 75.

- 859 - Steepness of the refractory period function (α_R): Set to 100.

860

861 **Data and materials availability**

862 Python codes are available on Github (<https://github.com/MignotLab/SpatialPatternsCollectives>).

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Competing interests Authors declare that they have no competing interests.

Additional information

Supplementary Information is available for this paper (Supplementary Text, figs. S1 to S9, tables S1 and movie S1 to S8).

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