

# Social Evolution of Spatial Patterns in Bacterial Biofilms: When Conflict Drives Disorder

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**ABSTRACT:** A key feature of biological systems is the emergence of higher-order structures from interacting units, such as the development of tissues from individual cells and the elaborate divisions of labor in insect societies. Little is known, however, of how evolutionary competition among individuals affects biological organization. Here we explore this link in bacterial biofilms, concrete systems that are well known for higher-order structures. We present a mechanistic model of cell growth at a surface, and we show that tension between growth and competition for nutrients can explain how empirically observed patterns emerge in biofilms. We then apply our model to evolutionary simulations and observe that the maintenance of patterns requires cooperation between cells. Specifically, when different genotypes meet and compete, natural selection favors energetically costly spreading strategies, like polymer secretion, that simultaneously reduce productivity and disrupt the spatial patterns. Our theory provides a formal link between higher-level patterning and the potential for evolutionary conflict by showing that both can arise from a single set of scale-dependent processes. Moreover, and contrary to previous theory, our analysis predicts an antagonistic relationship between evolutionary conflict and pattern formation: conflict drives disorder.

**Keywords:** sociobiology, cooperation, self-organization, pattern formation, *Pseudomonas aeruginosa*.

## Introduction

The study of group behavior is dominated by two complementary but largely distinct traditions. One focuses on the evolution of cooperation and group-level traits, and it asks how these factors remain stable in the face of evolutionary conflict at lower levels of selection (Hamilton 1964; Leigh 1991; Keller 1999; Lehmann and Keller 2006). Researchers of social insects (Bourke and Franks 1995; Ratnieks et al. 2006), vertebrates (Hatchwell and Komdeur

2000; Griffin and West 2003), and, increasingly, microbes (Crespi 2001; Foster et al. 2007; West et al. 2006, 2007; Nadell et al. 2009) have made great bounds in our understanding of cooperation, repeatedly finding evidence for conflict-resolution mechanisms such as kin selection and enforcement. The other tradition focuses on organization and asks how social groups, which contain many distinct individuals, are able to produce higher-level structures and patterns (Anderson and Franks 2001; Beshers and Fewell 2001; Kaitala et al. 2001; Theraulaz et al. 2003; Sumpter 2006; Couzin 2007). This tradition reveals how sophisticated group properties emerge from simple low-level behaviors, including the organization of insect foraging trails and societies (Detrain et al. 1999; Theraulaz et al. 2002, 2003; Couzin 2007), herding and shoaling in vertebrates (Couzin et al. 2005), and multicellular development (Salazar-Ciudad and Jernvall 2002; Jiang et al. 2004; Chu et al. 2006; Maini et al. 2006; Nelson et al. 2006).

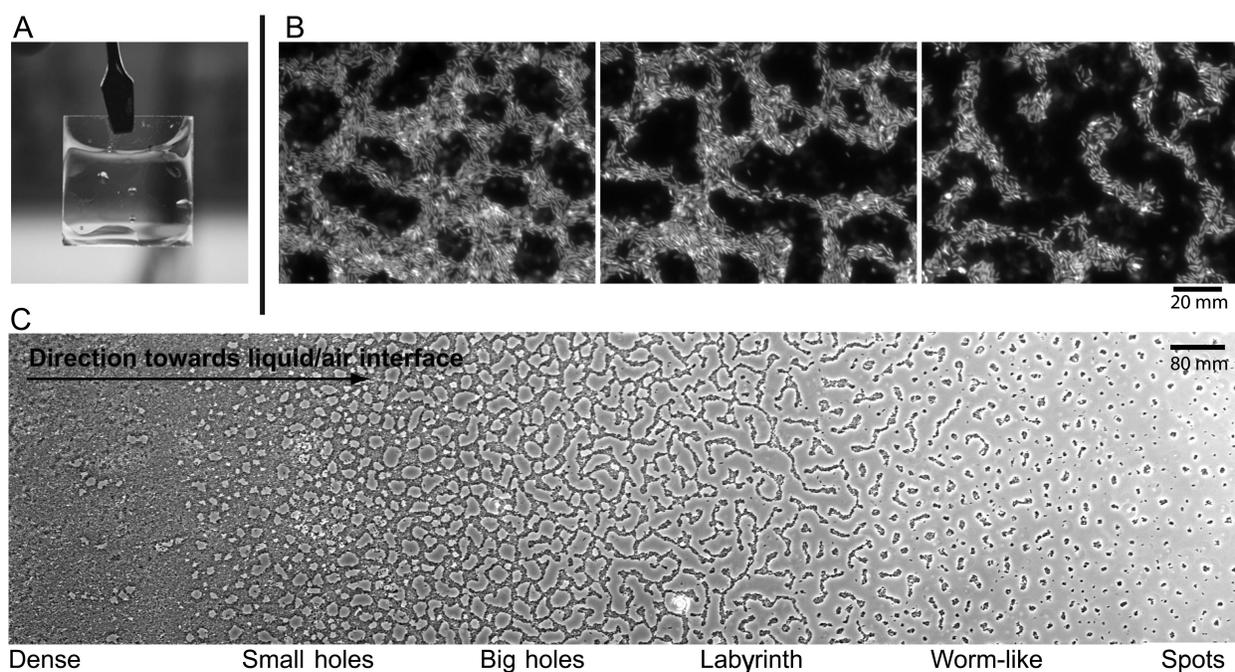
Some important links exist between conflict and cooperation on the one hand and higher-level patterns on the other. A series of studies have looked at social games on grids (e.g., Hauert and Doebeli 2004; Werfel and Bar-Yam 2004), including the famous Prisoner's Dilemma (Nowak and May 1992). Here, cooperators and defectors interact and evolve on a lattice, often driving striking higher-level dynamics ranging from simple patchiness to the emergence of fractals (Nowak and May 1992). The prediction that conflict can generate patterns finds further support in the existence of irregular patches of different bacterial genotypes when there is competition with toxins known as bacteriocins (Kerr et al. 2002; Reichenbach et al. 2007). Nevertheless, the formal links between evolutionary conflict on the one hand and emergent patterning on the other remain poorly understood.

Here we investigate the relationship between evolutionary conflict in social groups and higher-level patterns in a concrete biological system: bacterial biofilms. Surface-attached groups, known as biofilms, are central to micro-

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**Figure 1:** Spatial patterns in biofilms of *Pseudomonas aeruginosa*. Biofilms were cultivated on glass coverslips submerged in inoculated liquid medium. *A*, Coverslips were removed after 24 h to reveal a robust biofilm. *B*, Fluorescent microscopy of yellow fluorescent protein–labeled biofilm shows cells in spatial patterns with holes, labyrinths, and wormlike shapes. *C*, Continuous variation of spatial patterns across the surface of the coverslip is produced by the systematic variation of nutrient concentration. This image is a montage of four contiguous phase-contrast microscopy images.

bial life and have implications for many human activities (Kolter and Greenberg 2006), including persistent infections and antibiotic resistance (Costerton et al. 1999). It has recently been realized that one must consider processes at multiple scales of interaction to understand biofilm evolution (Kreft 2004; West et al. 2006; Xavier and Foster 2007; Nadell et al. 2008). In addition, and like so many social groups, biofilms display high-level patterns and structures, such as mushroom shapes (Klausen et al. 2003*b*), as well as regular patterns (Thar and Kuhl 2005), which have been implicated in resistance to antimicrobials (Davies et al. 1998) and nutrient influx (Costerton et al. 1994). However, the relationship, if any, between such patterns and the evolution of cooperation in biofilms remains unclear.

### Results and Discussion

Our goal is to develop an explicit and mechanistic model that formalizes the links between emergent pattern formation and the evolution of cooperation and conflict. For realism and clarity, we based our model on a simple experimental system from our laboratory: early biofilm development in *Pseudomonas aeruginosa* biofilms. However,

the model should be broadly applicable to populations of cells growing on a surface.

#### *Empirical Basis for the Model*

We observed pattern formation in biofilms in a simple assay, using a glass coverslip (25 mm × 25 mm; VWR, Pittsburgh, PA) placed in a tilted position in liquid medium inoculated with a low density of *P. aeruginosa* cells (2.5 mL of Triptone broth inoculated with cells at an optical density of 0.0025 in six-well plates; BD, Franklin Lakes, NJ). This setup was incubated for 24 h at room temperature in the absence of any agitation. The coverslip was then extracted and a dense band of biofilm was visible to the naked eye parallel to the air-water interface (fig. 1*A*). Microscopy revealed regular spatial patterns on the regions located on both the upper and lower edges of the band. Cells growing attached to the glass surface experience a nutrient concentration that systematically varies with depth; this produced a sparseness of patches that increases for locations away from the center of biofilm band. The biofilm structure also displayed a progression of regular shapes over hundreds of micrometers that shifts through hole, labyrinth, and spot patterns (fig. 1*B*). This progression of patterns within a single coverslip experi-

ment is visible from a wider field of view, as observed in a montage of lower-magnification images (fig. 1C).

### Mathematical Model Formulation

Turing provided a mathematical formulation to explain how biological spatial patterns reminiscent of those we observe in our biofilms can emerge from simple dynamics (Turing 1952). Turing considered a system of two chemical species with different diffusivities that react with each other, and he found that under some conditions, the system's fixed points—states that are linearly stable when spatial structure is neglected—become unstable to spatially heterogeneous perturbations. These so called “Turing instabilities” lead an initially homogeneous system to spatial structure. Similar mechanisms, Turing hoped, would provide a general mathematical foundation of biological organization (Allaerts 2003). As we describe below, Turing instabilities per se cannot explain the patterns we observe in our experiments. Our system is stable against the structural instabilities required to lead a system to patterns in Turing analysis. Nevertheless, we can explain the observed biofilm patterns by a mechanism that, like Turing's, has two components. However, only one is a diffusible species; the other is the cell population.

We describe cell-growth kinetics with the expression

$$\frac{\partial X}{\partial t} = \mu \frac{S}{S + K} X - mX. \quad (1)$$

The first term on the right-hand side represents growth of  $X$  (biomass concentration at the surface) following Monod kinetics (Monod 1949), where  $\mu$  is the maximum specific growth rate of bacteria,  $S$  is the concentration of growth-limiting nutrient, and  $K$  is the half-saturation constant. The second term is commonly used to represent all losses in biomass, including decay or cell-maintenance costs (Sinclair and Topivala 1970). The concentration of nutrient at the surface is governed by

$$\frac{\partial S}{\partial t} = Q(S_0 - S) - \frac{\mu S}{YS + K} X, \quad (2)$$

where  $S_0$  is the concentration of nutrient in the liquid above the surface,  $Q$  is the rate of mass transfer between liquid and surface, and  $Y$  is the yield of nutrient to biomass. Nutrient influx into the biofilm is driven by the concentration difference between bulk liquid and surface. Analysis of this system (detailed in the appendix in the online edition of the *American Naturalist*) reveals a single stable fixed point, here written in dimensionless form as

$$\begin{aligned} s^* &= k \frac{f}{1 - f^2} \\ x^* &= \frac{1 - s^*}{\phi^2 f}, \end{aligned} \quad (3)$$

where  $s = S/S_0$  and  $x = X/\rho$ , and asterisks denote that the value is at the fixed point. The parameter  $\rho$  represents the maximum concentration of biomass at the surface. Equation (3) reveals that to know the steady state of the system ( $s^*$ ,  $x^*$ ), one needs only three dimensionless parameters:  $k = K/S_0$ ,  $f = m/\mu$ , and the Thiele modulus,  $\phi^2 = \mu\rho/YQS_0$ , a group that quantifies the balance between consumption and transport of the nutrient. The existence of a single stable fixed point for any condition described by  $k$ ,  $f$ , and  $\phi^2$  explains that the biofilm will grow until its biomass reaches a steady state concentration. The next step is to determine whether that steady state has any spatial structure.

We first applied a traditional Turing instability analysis to our model whereby we assumed that cells and nutrient both spread on the surface by diffusion. However, contrary to the system originally analyzed by Turing (1952), our system is linearly stable against any spatial perturbations: no spatial patterns are predicted from Turing's analysis. Our analysis followed the formulation proposed by Murray (2004); it is detailed in the appendix.

An alternative way to model cell spreading on a surface was proposed by Dockery and Klapper (2001). A central assumption of their model is that the biofilm occupies space in patches of constant density,  $\rho$ . This is a reasonable assumption here, since the coverslip surface is in fact covered by cell patches of equal density (fig. 1B). Formally, Dockery and Klapper (2001) assumed that bacteria spread according to Darcy's law, whereby cell growth and division increase mechanical pressure locally according to

$$-\lambda \nabla^2 P = \frac{1}{X} \frac{\partial X}{\partial t}. \quad (4)$$

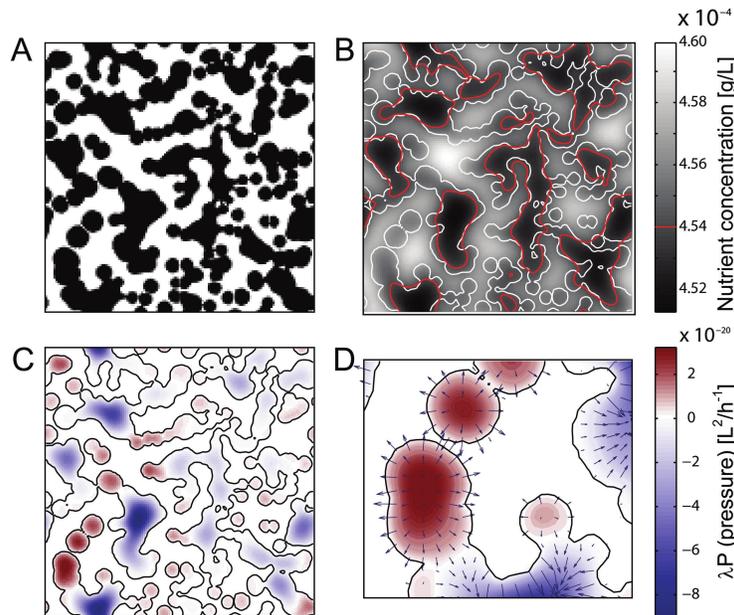
Pressure causes expansion of the biofilm front with velocity

$$\vec{v} = -\lambda \vec{\nabla} P \quad (5)$$

where  $\lambda$  is Darcy's constant for the biofilm and is equal to  $\partial X/\partial t$ , as set by equation (1). Growth is a function of the local nutrient concentration, which in turn is determined by the partial-differential equation

$$\frac{\partial S}{\partial t} = Q(S_0 - S) - \frac{\mu S}{YS + K} X + D \nabla^2 S, \quad (6)$$

where  $D$  is the diffusivity of the nutrient. Dockery and



**Figure 2:** Details on the two-dimensional simulations. *A*, Any given spatial distribution of cells (surface occupied by cells is black) causes small heterogeneities in nutrient concentration (*B*). The growth rate of any cell within the biofilm then becomes a function of the local nutrient that cell is experiencing. The population spreads by growing only in regions where this concentration is enough to overcome maintenance requirements. The isoconcentration line (red) represents the nutrient limit computed from  $s^*$ ,  $4.54 \times 10^{-4} \text{ g L}^{-1}$  in this case. *C*, Cumulative effect of small differences in growth rate generates uneven pressure. The velocity at which the population front advances (blue arrows in *D*) is determined from the gradient of the pressure. Computational domains in *A–C* represent an area of  $100 \times 100 \mu\text{m}^2$  (*D* is a detail from *C*).

Klapper (2001) showed through linear analysis how the advancing front of a biofilm can develop fingering instabilities. This fingering process is essential in our case, since it is the symmetry-breaking event that causes the distortion of initially round colonies.

#### *How the Patterns Emerge from Competition for Nutrients*

We pursued our analysis computationally to determine the spatial structures of the steady state, using the formulation introduced by Dockery and Klapper (2001). The model describes populations of cells as occupying the surface in patches with a constant cell density  $\rho$ . Instead of assuming that cells travel along the surface through diffusion, the model calculates the pressure generated by cell growth and division. This means using the following set of partial-differential equations:

$$\begin{aligned} 0 &= Q(S_0 - S) - \frac{\mu}{Y} \frac{S}{S + K} X + D\nabla^2 S, \\ -\lambda \nabla^2 P &= \frac{1}{X} \frac{\partial X}{\partial t}, \\ \vec{v} &= -\lambda \vec{\nabla} p. \end{aligned} \quad (7)$$

Note that the first equation describes nutrients at a steady state of reaction-diffusion. This derives from the assumption commonly used in biofilm models that reaction-diffusion is much faster than cell growth and division (Xavier et al. 2005). The second and third equations use Darcy flow to describe dynamics of the cell population. The model is made dimensionless, producing the equations

$$\nabla^2 S - \phi^2 \frac{s}{s + k} x + (1 - s) = 0, \quad (8)$$

$$-\nabla^2 p = \frac{s}{s + k} - f, \quad (9)$$

$$\vec{v} = -\lambda \vec{\nabla} p, \quad (10)$$

where  $s$ ,  $p = P\lambda/D$ , and  $v = v/(QD)^{1/2}$  are the dimensionless forms of nutrient concentration ( $S$ ), pressure ( $P$ ), and velocity ( $\vec{v}$ ), respectively. We solved this model numerically (Dockery and Klapper 2001) on an orthogonal grid with  $129 \times 129$  grid nodes and cyclic border conditions. The solver was implemented in Java and used an iterative cycle with the following operations:

1. For a given biomass distribution (fig. 2*A*), solve the

nutrient equation to steady state using the full-approximation storage multigrid method (Press et al. 1997).

2. With the nutrient concentrations (fig. 2B), solve the equation for pressure, again using the multigrid solver.

3. Using the pressure obtained (fig. 2C), determine velocity vectors by computing the gradient of the pressure.

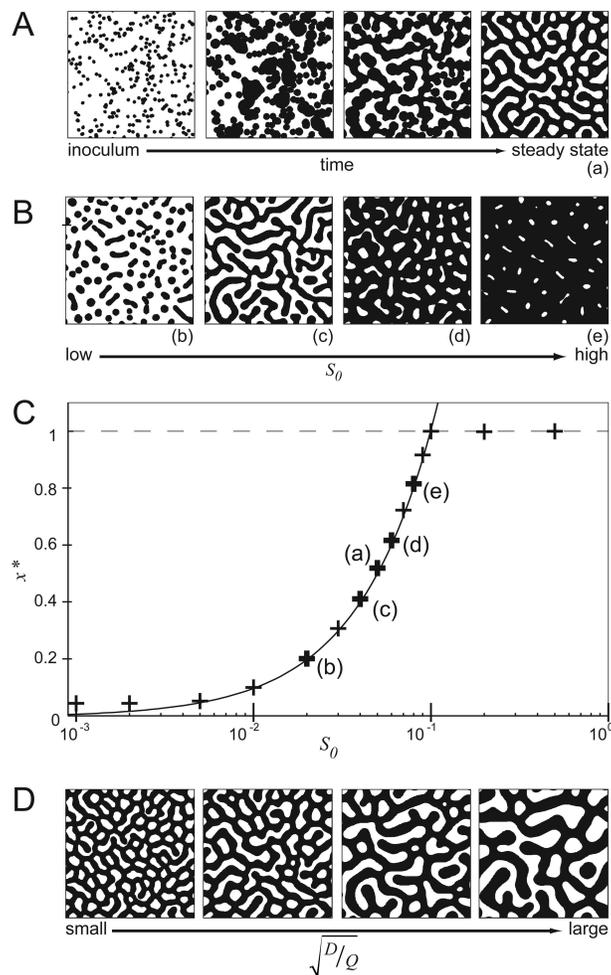
4. Use velocities (fig. 2D) to advance the biomass front using a level set function (Osher and Fedkiw 2003).

5. Advance time and return to operation (1) with the updated biomass distribution.

We started simulations from an initial state that represented a surface originally inoculated with individual cells, placed at random locations, that together occupied 1% of the surface (fig. 3A). In a typical simulation, at the

beginning, the inoculation sites develop into circular colonies by spreading outward. As the population grows, the nutrient concentration at the surface decreases so that it no longer sustains fast growth, and the advancement of these colonies begins to slow. Growth becomes heterogeneous in space, causing the circular patches to deform due to fingering. The system stabilizes into its final spatial structure when biomass at the surface is such that the nutrient influx is only enough to satisfy the biomass maintenance requirements. At the end, these simulations reveal the striking structural patterns observed in our biofilm experiments (fig. 3B).

We then performed the simulations at various parameter values and observed that the amount of biomass at steady



**Figure 3:** Mathematical modeling explains nutrient-dependent biofilm patterns. Patches occupied by cells are represented in black, whereas white represents empty surface locations. *A*, Time series from a simulation shows emergence of patterns, starting from the inoculum until steady state. *B*, Patterns at steady state for varying nutrient concentration ( $S_0$ ). *C*, Amount of biomass at steady state ( $x^*$ ) depends on  $S_0$ ; solid line represents analytical solution (eq. [3]), crosses represent two-dimensional simulations, dashed line at  $x^* = 1$  represents limit at which two-dimensional simulations predict complete surface occupation. *D*, Scale of patterns is set by  $(D/Q)^{1/2}$ , which has dimensions of length.

state matches that calculated by the spatially homogenous model (comparison in fig. 3C). These calculations show that equation (4) is still valid to describe the steady state even in the presence of spatial structure. Importantly, it reveals that nutrient availability can explain the changing density of patterns observed (fig. 1C).

These simulations also make the interesting prediction that the scale of the patterns is not defined by the amount of biomass at steady state. Rather, the length scale of patterns is set by  $h = (D/Q)^{1/2}$ , the square root ratio of nutrient diffusivity and its external mass transfer. This prediction means that, all else being equal, changes in  $h$  will change the size of the patterns without affecting total biomass productivity, an effect that resembles zooming in on the patterns (fig. 3D).

#### *Empirical Support for the Model*

The observed patterns and their progressions (fig. 1C) are consistent with the effects of nutrient conditions: the biofilm band is denser at an intermediate distance of the air-water interface, where conditions are most likely to be optimal due to intersecting gradients of oxygen and limiting nutrients in the liquid medium. In addition, we performed experiments that altered the inclination of the coverslip in the media. The results showed that the width of the band decreased with the tilting angle (fig. 4A). This is consistent with the role of nutrient gradients along the depth of the liquid in generating the pattern progression shown in figure 1C. In addition to nutrient effects, another key factor for pattern formation in our model is that there is limited surface motility beyond that caused by cells pushing one another as they grow and divide. Experiments with four fluorescently labeled constructs of the same strain reveal significant clustering by color (fig. 4B–4G), which again is consistent with the assumptions of the model. Another test of our model would be to examine the predicted scaling law (fig. 3D); however, this requires manipulating either the diffusivity of the growth-limiting substrate or its external mass transfer, neither of which we can do in our experimental system. Nevertheless, general support of the scaling prediction comes from the observation of similar patterns at the millimeter scale in marine biofilms (Thar and Kuhl 2005) and at the meter scale in arid vegetation (Rietkerk et al. 2004).

Can we expect the observed patterns to be general to all biofilms, or do they instead require specific conditions such as our tilted-coverslip assay? Our model predicts that we should observe patterns in any other biofilm system where surface occupation is not total and where there is sufficiently limited movement by cells. What makes our empirical system uniquely suited is that, by providing a gradient of nutrients along the tilted coverslip surface, we

ensure that there are always some locations where conditions are suitable for pattern formation.

#### *Patterns Emerge from a Combination of Nutrient Limitation and Mechanical Pushing*

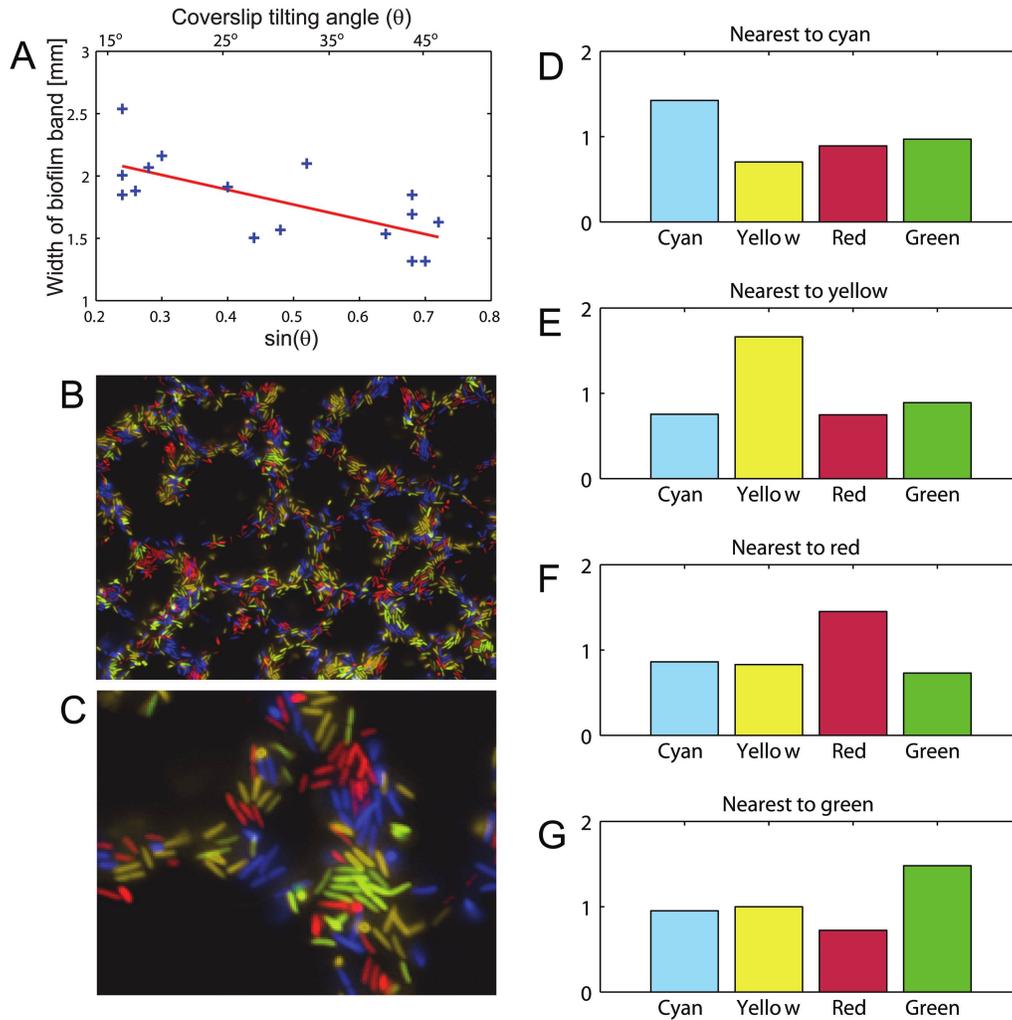
Pattern formation in biofilms can be understood in terms of two key processes. First, cells exert a negative influence on each other by lowering local nutrient concentration. The scale of this interaction is set by the properties of nutrient transport, and its strength is set by the properties of nutrient consumption. At the same time, cell growth and division produce mechanical pressure, causing the colonies to spread. This is an interaction among neighbors that acts at a range closer than that of nutrient conflict. In the end, the frequency of the spatial patterns increases to levels such that it becomes effectively homogeneous at the length scale set by nutrient diffusion.

The two processes behind pattern formation can also be characterized as opposing social effects. Whereas nutrient consumption is a competitive interaction, mechanical pushing is cooperative. Cells can benefit from receiving a push as they increase their growth rate by traveling up the gradients of nutrient concentration, as we analyzed previously (Xavier and Foster 2007). A key finding of our analysis is that the patterns in our system arise from the very same processes that were central to the evolution of cooperation and conflict in our previous models of biofilms: regional competition for nutrients and cooperative pushing of cells into nutrient-rich regions.

#### *Cooperation, Conflict, and Pattern Formation*

The conclusion that a single set of processes—nutrient limitation and pushing—both drives natural selection and generates emergent spatial patterns in biofilms leads to at least two interesting corollaries. First, it suggests that the scale of patterns observed in nature can be used to infer the evolutionarily relevant scales of competition within groups of unrelated social organisms (an observation that we leave open for now). A second corollary, and our focus here, is that an evolutionary response to the mechanisms that cause patterns has the potential itself to affect patterning. We therefore evaluated the potential for an interaction between evolutionary conflict and pattern formation among strains with different spreading strategies and under different social conditions.

We proceed here with theoretical approaches, as empirical approaches proved to be intractable. Specifically, we performed preliminary experiments to search for mutants with different levels of surface motility that could be used to study competition. These included several mutants in flagella and pili genes that affect the structure of *P. aeru-*



**Figure 4:** A, Tilting assay confirms that biofilm width decreases with increased coverslip inclination. Blue crosses represent data from independent experiments, and the red line shows the best linear fit to the sine of the tilting angle. B, C, Biofilms composed of *Pseudomonas aeruginosa* strains labeled with cyan fluorescent protein, yellow fluorescent protein, green fluorescent protein, and DsRedExpress were imaged (four color channels overlaid in B; detail in C) and processed computationally to obtain coordinates of individual cells. D–G, Cells are located next to neighbors of the same color more than would be expected by random positioning using a weighted measurement defined as  $n_{ij} = (N_{ij}/N_i) \times (N_j/N_T)$ . Here,  $i$  represents the color of a focal cell, and  $j$  is the color of its nearest neighbor.  $N_{ij}$  is the number of cells of color  $i$  whose nearest neighbor has color  $j$ ,  $N_i$  is the total number of cells of color  $i$ , and  $N_T$  is the total number of cells in the image analyzed.

*ginosa* biofilms in flow cells for pattern formation (Klausen et al. 2003a). However, none of the mutants tested were capable of producing biofilms in our coverslip setup. We also tested mutants that overproduce extracellular slime (mucoid variants) under some conditions, because we hypothesized that slime production may result in increased spreading on the surface. However, like the wild type, these mutants do not appear to produce significant levels of polymer under our assay conditions, and they made patterns that were no different from those of the wild type (not shown).

We therefore focus on simulations, with the goal of establishing a formal theoretical link between the evolution of competition and pattern formation. To allow generality of the spreading mechanism, we considered different values of the maximum cell density for each strain as a proxy for cell spreading. A decrease in cell density might be achieved by polymer secretion, as in the vertical-slice simulation of Xavier and Foster (2007), where bacterial cell separation in biofilms is mediated by the extracellular polymers. However, it could also represent increased motility and movement across the surface, as long as patches

of different strains maintain a low level of mixing. In our model, then, a strain with lower density spreads across the surface more for the same number of cell divisions, all else being equal.

*Among-Group Competition.* We first consider the case of groups that are formed from a single cell or from cells of identical genotype, so that all evolutionary competition is among groups (we also assume no mutation; West et al. 2006). In this case, groups are formed by either a non-spreader strain or a spreader strain. Central to our analysis is that spreading carries a finite metabolic cost. This is biologically realistic for bacteria in the cases of both polymer excretion (Klausen et al. 2003b) and motility (Harshey 2003). In our model, two strains that differ in spreading ability, a fast spreader (F) and a slow spreader (S), will differ in cell density per surface area such that  $\rho_F < \rho_S$ . If we assume, as previously, that kinetics of nutrient uptake per biomass is maintained (Xavier and Foster 2007), then the cost of spreading is the result of a lower yield of biomass produced per mass of nutrient consumed, that is,  $Y_F < Y_S$ . Consequently, the maximum specific growth rate of the fast spreaders is lower than that of slow spreaders and is expressed as

$$\mu_F = \frac{Y_F}{Y_S} \mu_S. \quad (11)$$

When nutrients are plentiful, growth is exponential and the competition is won by the strain with the highest maximum specific growth rate. In these conditions, spatial structure has no effect, and therefore slow spreaders outgrow fast spreaders.

When nutrients are growth limiting, spatial structure becomes important. However, in the case where competition is among biofilms, the outcome of each competition is determined by the amount of biomass produced separately by each type. That amount is given by  $x^*$  in equation (3); this allows the evolutionary dynamics to be followed analytically. The number of cells of each strain at the end of each competition is

$$\frac{X_F^*}{X_S^*} = \frac{Q[(S_0 - \{K/[(\mu_F/m) - 1]\}]/(m/Y_F)]}{Q[S_0\{K/[(\mu_S/m) - 1]\}]/(m/Y_S)]} \approx \frac{Y_F}{Y_S}. \quad (12)$$

The frequency of fast spreaders in the total population after one competition ( $w'$ ) is a function of its frequency before that competition ( $w$ ) as follows:

$$w' = \frac{1}{1 + [(1 - w)/w](Y_S/Y_F)}. \quad (13)$$

Recalling that  $Y_F < Y_S$ , equation (13) states that the pro-

portion of the fast strain in the population always decreases ( $w' < w$  for any  $w$ ) and that successive rounds of competition lead to its extinction. This is shown in figure 5A. In conclusion, if competition is among biofilms, the slower spreader always wins against the faster one, and consecutive rounds of competition always result in extinction of the faster spreader.

*Within-Group Competition.* What happens if multiple genotypes colonize each surface so that strains meet each other within each group? In the absence of nutrient limitation, that is, in conditions for which patterns do not form, slower spreaders still prevail. This happens because the competition is set purely by differences in growth rate (eq. [11]). When strains mix under diffusion limitation, however, the interaction becomes more complex and requires simulation. We therefore extended our framework to analyze the effect of mixing strains. Two-strain competitions were implemented by extending the model to include multiple species (Alpkvist and Klapper 2007).

We considered the opposite extreme to the single-strain case, in which each surface is seeded by many strains that are randomly sampled from the population so that average genetic relatedness within each group is close to 0 (Gilbert et al. 2007). In this case, the frequency of the fast-spreader genotype is then identical in each competition (equal to the population mean), and the evolutionary outcome is determined solely by competition within each group (West et al. 2006). Figure 5B shows the result for a specific case ( $Y_F/Y_S = 0.67$  and  $\rho_F/\rho_S = 0.5$ ). These results show that within-group competition can have outcomes that are opposite those of the low-mixing case (among-groups competition). Fast spreaders are now capable of invading from any initial frequency, in spite of spreading costs. Accompanying that invasion is a strong decrease in total group productivity (fig. 5C).

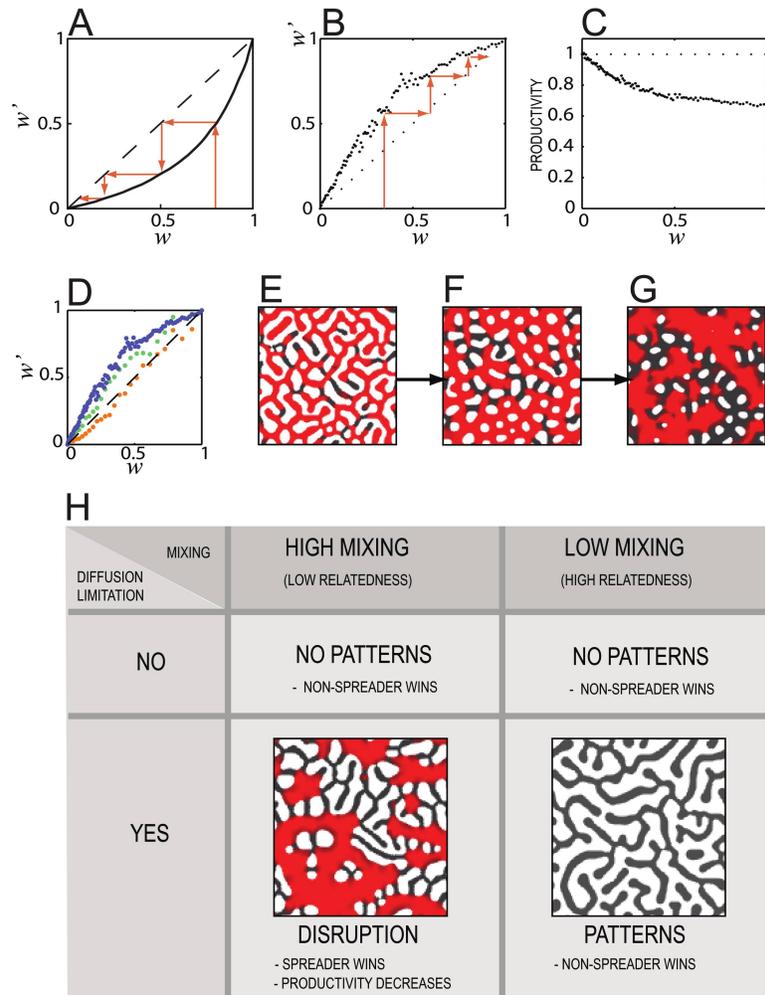
The plot shown in figure 5B–5C is a specific realization for the parameters  $Y_F/Y_S = 0.67$  and  $\rho_F/\rho_S = 0.5$  that has been chosen for illustration. We can, however, generalize these conclusions beyond these parameters. We first define the cost of spreading ( $c$ ) as the ratio of the density (a parameter inversely related to spreading) and the yield (inversely related to the cost):

$$c = \frac{\rho}{Y}. \quad (14)$$

For the fast strain to spread more than the slow strain, their costs must fulfill

$$c_F < c_S. \quad (15)$$

If this were not the case, the cost of spreading more would



**Figure 5:** Evolutionary analysis, where  $w$  represents the proportion of spreaders in the population before a competition, and  $w'$  represents the same proportion at its end. Within-group conflict can lead to evolution of spreading, disrupting patterns. *A*, When competition is among groups (low mixing, clonal groups), successive competition rounds (arrows) lead to the extinction of spreaders because, due to the cost of spreading, they are less productive. *B*, When competition is within group (high mixing, zero relatedness), the system is no longer analytically tractable, so we applied simulations. Here, the outcome is reversed: spreaders invade (arrows) in spite of the cost of spreading. However, invasion results in a decrease in the total group productivity (*C*), because resources are spent in costly spreading. *D*, Evolutionary dynamics in conditions of nutrient limitation and high mixing (within-group competition). Simulations for a range of costs show that fast spreaders win over slow spreaders, in spite of the cost of spreading and the existence of an equilibrium between  $c_F = 754$  and  $c_S = 565$  (blue:  $c_F = 424$  and  $c_S = 318$ ; green:  $c_F = 565$  and  $c_S = 424$ ; orange:  $c_F = 754$  and  $c_S = 565$ ). Selection of fast spreaders in conditions of nutrient limitation and high mixing leads to the disruption of patterns. Steady states from competitions started at  $w = 0.50$ , with  $c_F = 424$  and  $c_S = 318$  (*E*),  $c_F = 565$  and  $c_S = 424$  (*F*), and  $c_F = 754$  and  $c_S = 565$  (*G*). In each panel, the faster spreader is represented in red and the slower spreader is represented in gray. *H*, Summary of evolutionary outcome: an interaction between low nutrients and strain mixing (low genetic relatedness) selects for strains that spread (red) and do not make patterns (bottom left quadrant) and reduces biofilm productivity in an evolutionary tragedy of the commons. Pattern-forming strains (nonspreaders) evolve in all other cases, although pattern formation occurs only under nutrient limitation.

be higher than the benefit of obtaining extra nutrients. When equation (15) is satisfied, however, we observe from simulation that our conclusion is indeed general: fast spreaders always invade, irrespective of the cost (fig. 5*D*). As a note, our simulations are capable of following this evolution only up to the point that the strain covers the

surface completely and our simulations can no longer predict spreading advantages. Simulations with these evolved fast spreaders show no patterns.

In addition, further competitions show that strains with progressively more spreading can invade strains with lower spreading capabilities. This leads to a loss of pattern for-

mation (fig. 5E–5G). Coupled with the loss of patterns, the rise of spreaders results in a reduced group productivity when compared with the ancestral, slower-spreading strain (fig. 5C), which had previously succeeded in among-group competitions. With mixing, strains that spread better gain a selfish benefit but spend a shared resource (the nutrient). In doing so, they reduce total group productivity, leading to more resource spending and even lower productivities. This situation is akin to an evolutionary tragedy of the commons, a scenario that frequently arises when multiple users exploit the same resource (Hardin 1968; Kerr et al. 2006; Rankin et al. 2007).

### Conclusions

Scale-dependent competition is central to both the study of social conflicts, where it defines the potential for useful cooperation among relatives (Wilson et al. 1992; Queller 1994; Perrin and Lehmann 2001; West et al. 2002; Gardner and West 2004), and self-organization, where a mix of positive and negative feedback can cause the emergence of higher-level patterns (Turing 1952; Salazar-Ciudad and Jernvall 2002; Theraulaz et al. 2002, 2003; Jiang et al. 2004; Rietkerk et al. 2004; van de Koppel et al. 2005; Chu et al. 2006; Maini et al. 2006; Sumpter 2006). Our study suggests that this association can be more than superficial. We saw that the very same scale-dependent interactions—nutrient competition and mechanical pushing—that drive the occurrence of spatial patterns can also generate natural selection for competition among strains. In simulations containing many unrelated strains, we find that natural selection favors spreading strategies that allow strains to compete within groups (fig. 5H). This finding agrees with those of our previous studies, that increased bacterial cell spreading by polymer secretion can be favored in biofilms in spite of its cost (Xavier and Foster 2007). Moreover, we show here that such costly strategies can simultaneously disrupt higher-order structure. In addition to the energetic costs of spreading that we analyzed here, conflict may incur additional costs: a loss of structure in biofilms has been found to correlate with increased susceptibility to antibiotics (Davies et al. 1998; Stewart and Costerton 2001).

To conclude, we provide a formal link between higher-level patterning and the potential for evolutionary conflict in social systems by showing that both can arise from a single set of scale-dependent processes. This close association, however, is an antagonistic one: we also find that the expression of conflict will disrupt the very patterns that might otherwise indicate its nature. A close analogy can be found in multicellular development. Nelson et al. (2006) showed that branching patterns during mammary development result from the combination of mechanical pushing by cells and growth arrest induced by secreted

inhibitory morphogens. Analogous to our spreader strategy, this development can be disrupted by cancerous cells that ignore the inhibitory signal and overgrow the non-cancerous cells, resulting in unstructured tissue. In contrast, the emergence of fractal patterns in the lattice simulations of Nowak and May (1992) requires an interplay between both defector and cooperator strategies; a lattice of only cooperators would presumably lack structure. The key difference between these lattice models and our own, therefore, is that in our system—as in multicellular development—cooperators produce patterns when alone. It is these cooperative patterns that are susceptible to disruption by the evolution of defectors. However, the potential for secondary patterns to then emerge from the interaction of cooperators and defectors (Nowak and May 1992) suggests that the relationship between conflict and patterns is a nontrivial one.

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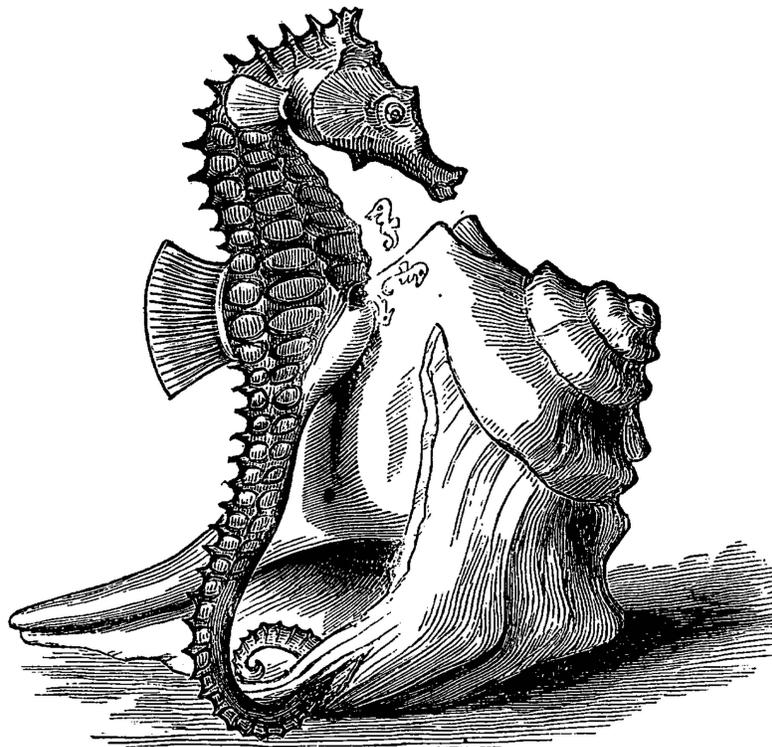
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Sea horse (*Hippocampus hudsonius*) from “The Sea-Horse and its Young” by Rev. Samuel Lockwood (*American Naturalist*, 1867, 1:225–234).