The ecological basis of morphogenesis: branching patterns in swarming colonies of bacteria

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Abstract
Understanding how large-scale shapes in tissues, organs and bacterial colonies emerge from local interactions among cells and how these shapes remain stable over time are two fundamental problems in biology. Here we investigate branching morphogenesis in an experimental model system, swarming colonies of the bacterium Pseudomonas aeruginosa. We combine experiments and computer simulation to show that a simple ecological model of population dispersal can describe the emergence of branching patterns. In our system, morphogenesis depends on two counteracting processes that act on different length-scales: (i) colony expansion, which increases the likelihood of colonizing a patch at a close distance and (ii) colony repulsion, which decreases the colonization likelihood over a longer distance. The two processes are included in a kernel-based mathematical model using an integro-differential approach borrowed from ecological theory. Computer simulations show that the model can indeed reproduce branching, but only for a narrow range of parameter values, suggesting that P. aeruginosa has a fine-tuned physiology for branching. Simulations further show that hyperswarming, a process where highly dispersive mutants reproducibly arise within the colony and disrupt branching patterns, can be interpreted as a change in the spatial kernel.

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1. Introduction

Branching patterns are common in multicellular tissues and organs. The branches and roots of trees (De Smet and Jürgens 2007), the circulatory system and lungs in vertebrates (Metzger et al 2008), the branched colonies of corals (Helmuth et al 1997) and the networks of slime molds (Tero et al 2010) are some examples across Eukaryotic taxa (figure 1(A)). Branching tissues have long fascinated biologists and mathematicians alike, and much progress has been made in revealing mechanistic details (Affolter et al 2009, Ochoa-Espinosa and Affolter 2012). Less attention has been paid to identifying general principles of their morphogenesis. Branched structures increase the area for transfer of substances into and away (e.g. nutrients and wastes, respectively) from body sites. Consequently they enable multicellular organisms to achieve sizes otherwise impossible. The genetic programs controlling tissue branching evolved under specific physical constraints to optimize resource transport while minimizing physiological cost to the organism (Sherman 1981). Tissue branching can therefore be considered an example of cooperation between cells, which is the hallmark of multicellularity. Multicellular organisms are mostly monoclonal, explaining why individual cells engage in altruistic behaviors (Crespi and Summers 2005). However, if (epi)genetic changes yield clonal diversification, natural selection may revert to the level of individual cells (Greaves and Maley 2012), in turn leading to pattern disruption. Cancerous tumors are one manifestation of this process and their unregulated growth destroys branching patterns (Cellurale et al 2012, Chiu et al 2012) (figure 1(B)). Understanding branching morphogenesis and disruption is therefore of great interest to both biology and medicine.

Here we investigate branching morphogenesis in a simple experimental system: swarming colonies of the bacterium Pseudomonas aeruginosa. P. aeruginosa is not a multicellular organism but has social traits resembling multicellularity, such as biofilm formation (Costerton et al 1999, O’Toole et al 2000, Xavier et al 2009), cell-to-cell communication (Davies et al 1998) and swarming motility (Kohler et al 2000, Rashid and Kornberg 2000, Xavier et al 2011, Du et al 2012). Swarming allows a colony to migrate collectively over soft agar surfaces and travel distances that are several orders of magnitude longer than their cell-length within a few hours. P. aeruginosa swarms can have flat, two-dimensional (2D) branches that are approximately 2–5 mm wide and less than 1 mm thick, with branching points typically approximately 1 cm from each other (figure 1(D), left panel). In a recent paper, we tested the stability of the branching patterns to new mutations arising within the population (van Ditmarsch et al 2013). We carried out experimental evolution by transferring swarming bacteria to a new plate repeatedly after each 24 h swarming. After only a few days we observed the emergence of hyperswarming mutants (figure 1(C)). The mutations made P. aeruginosa gain multiple flagella, which increased their dispersal ability. As a consequence the colonies lost their branching pattern (figure 1(D)).

Some previous models of bacterial colonies or biofilms rely on mechanistic details such as reaction-diffusion (e.g. Matsushita and Fujikawa 1990, Fujikawa and Matsushita 1991, Picoreanu et al 1998, Cohen et al 1999, Netotea et al 2009, Xavier et al 2009, Nadell et al 2010). Since the seminal work of Turing on morphogenesis (Turing 1952) it is well known that diffusion-reaction processes can generate spatial patterns in biology (Lander 2011). Colonies in other bacterial species show branching patterns that are indeed driven by diffusion-limited growth (Kawasaki et al 1997). In contrast, the branches in P. aeruginosa swarming are driven by different processes requiring active flagellar motility (Kohler et al 2000), cell–cell
Figure 1. Branching patterns are ubiquitous in nature, but pathologies of various kinds can lead to their disruption. (A) Examples of branching in multicellular organisms include rigid corals (i), tree branches (ii), lung networks in mammalians (iii) and networks in slime molds (iv). (B) Branching patterns can be disrupted by unregulated growth such as tumors, shown here in trees (i), mammary glands (ii) and corals (iii). (C) Evolutionary experiments with swarming colonies of the bacterium P. aeruginosa demonstrate how branching can be disrupted by evolution of highly dispersive mutants called hyperswarmer (van Ditmarsch et al 2013). (D) Wild-type P. aeruginosa have a single flagellum (left panel, inset shows electron microscopy of a single P. aeruginosa bacterium with one flagellum); the highly dispersive hyperswarmer mutants evolved in the lab have multiple flagella and their
Figure 1. (Continued) colonies have very distinct morphologies without branches (right panel, inset shows electron microscopy of two hyperswarmer mutants with multiple flagella each). Image credits: (A.i) Wikimedia Commons file ‘1991 coral-svalbard hg.jpg’; (A.ii) Bordalier Institute; (A.iii) Metzger et al (2008); (A.iv) Wikimedia Commons file ‘Physarum polycephalum plasmodium.jpg’; (B.i) Photo by Vicky Somma; (B.ii) Geneva Foundation for Medical Education and Research; (B. iii) Dr Jill Chiu, University of Honk Kong; (C, D) (van Ditmarsch et al 2013).

signaling (quorum sensing) (Xavier et al 2011) and, importantly, biosurfactants that disperse away from the colony and act as long-range signals (Caianza et al 2005, Tremblay et al 2007); see supporting video 1 (available from stacks.iop.org/NJP/16/015006/mmedia). Thus, 
P. aeruginosa branching resembles multicellular systems such as mammary branching which also require production and diffusion of morphogenic signals but where the cell patterning is driven by mechanical processes instead of diffusion (Nelson et al 2006). One possible way to model 
P. aeruginosa swarming is to aim for mechanistic realism, as was carried out successfully in a highly detailed multiscale model published recently (Du et al 2011). Another option, which we explore here, is to use a coarse-grained, phenomenological approach borrowed from ecology: dispersal kernels.

Swarming is a process of population dispersal. In ecological theory, spatial kernels are often preferred to diffusion equations as a way to model dispersal (Kot et al 1996). The main advantage is that kernel methods can accommodate a range of dispersal patterns (Lindström et al 2008, Lindstrom et al 2011), whereas diffusion assumes that dispersion is Gaussian. Furthermore, the combined effect of multiple processes acting simultaneously but with different strengths and at different length scales can be modeled by combining spatial kernels. For example, in many ecosystems the likelihood of colonization of a new patch increases with the number of colonized patches within a short distance but decreases with the number of patches colonized over a longer distance. This type of distance-dependent colonization, where there is a benefit of having close neighbors but a cost to being within a wider heavily populated region, can lead to spatial patterns similar to those of reaction–diffusion models (Rietkerk et al 2004). This can be modeled using spatial kernels such as the Laplacian kernel, which have a ‘Mexican hat’ shape where the sign is positive close to the center but becomes negative with distance.

Here, we apply the spatial kernel approach to model bacterial swarming and show that it can indeed produce branching patterns. We implement the model using a simple cellular automaton, called SIMSWARM, which simulates colony expansion using repeated iterations of a convolution algorithm similar to integro-difference equations (Kot et al 1996). We conduct novel swarming experiments to assess the length-scales of counteracting processes and we use these new experimental results to parameterize the SIMSWARM model. Our results demonstrate that the simple modeling approach can describe branching morphogenesis independently of the mechanisms involved. Further, our analysis suggests that differences in morphology of swarming colonies, such as in hyperswarmers that disrupt 
P. aeruginosa branching (van Ditmarsch et al 2013), may be attributed to changes in a spatial dispersal kernel.
2. Results and discussion

2.1. Ecological model of colony dispersal

We introduce a simple phenomenological model to describe the formation of branched colony shapes. The model uses a spatial kernel to represent the colonization of a patch, \( L(d) \), with respect to the colonization of patches at distance \( d \) to a focal point. The model then uses an integro-difference approach to calculate the rate of colonization of each patch in the system. In 2D the dynamics of colonization of a focal patch, \( N \), is given by

\[
N_{t+1} = \int_{-\infty}^{+\infty} L(d_{x,y})N_t(x, y) \, dx \, dy,
\]

where \( d_{x,y} \) is the distance between the focal niche and the niche located in coordinates \((x, y)\). In its discrete form, equation (1) can be implemented by a convolution of the distance kernel, \( L \), and a matrix representing the colonization of the ecosystem, \( N \). Dispersal patterns of ecological populations can take on several forms and the main advantage of the spatial kernel approach is that it allows implementing dispersal patterns of arbitrary shapes. A generic exponential equation for the kernel allows separating shape (kurtosis) from variance (Lindström et al 2008)

\[
L_+ (d) \propto 2^{-\left(d/d_1\right)^{h_1}}.
\]

In this equation, the exponent \( h_1 \) sets the shape. Note that if \( h_1 = 2 \), the dispersal is a Gaussian distribution and that for \( h_1 = 1 \) the dispersal follows a negative exponential (Lindström et al 2011). For convenience, we adopt exponents of base 2 rather than natural exponential base \( e \) used by Lindström et al (2008). By doing this, the biophysical meaning of the parameter \( d_1 \) becomes clear: \( d_1 \) is the distance (in units of length) where the colonization rate, \( L_+ (d) \), is half of its maximal value. We used the ‘+’ sign in this notation to highlight that the process is a positive contribution to colonization.

We now consider a second process that counteracts dispersal. We assume that the second process is, like equation (2), a function of the distance to the focal patch

\[
L_- (d) \propto 2^{-\left(d/d_2\right)^{h_2}}.
\]

This process implements negative effects such as crowding (Rietkerk et al 2004) or, in the case of \( P. \ aeruginosa \) swarming, repulsion mediated by colony-repelling surfactants (Tremblay et al 2007). As for \( d_1 \), \( d_2 \) has a biophysical meaning: it represents the distance (in units of length) at which the effect of repulsion effect decreases to half of its maximal value. The neighborhood of negative interaction is wider than the neighborhood of positive interaction (\( d_2 > d_1 \)). This creates a ‘Mexican hat’ kernel when both processes are added together (figure 2(B) inset). The exponents \( h_1 \) and \( h_2 \) (dimensionless parameters) may have different values to account for specific shapes in the positive and negative neighborhood influences.

The rate of colonization of a focal niche results from a spatial interaction kernel where the net contribution from a niche located at distance \( d \) is given by a weighted sum of \( L_+ \) and \( L_- \). The resulting spatial kernel is given by

\[
f(d) = b \times 2^{-\left(d/d_1\right)^{h_1}} - 2^{-\left(d/d_2\right)^{h_2}}.
\]
Figure 2. Swarming is modeled using a distance-dependent spatial kernel. (A) The rate of colonization of a focal patch (x) increases with the colonization within a close neighborhood (of radius $d_1$ represented in green) but decreases with the colonization of a wider neighborhood (of radius $d_2$ represented in red). (B) The spatial kernel used to calculate colonization rates (equation (4)) changes its shape depending on the value of the exponent $h$. Inset shows a 3D rendering of the kernel with $h = 10$.

Here, $b$ scales the strength of the positive process relative to the negative process. Figure 2(B) illustrates how the shape of $f(d)$ changes depending on the values of the shape parameter, the exponent $h$ (here we assumed $h_1 = h_2 = h$).

Intuitively, our model means that the rate of colonization of a focal patch in the system increases with the number of occupied patches within a close neighborhood but decreases with the occupation of a wider neighborhood. It should be highlighted that although the functional form proposed in equation (4) seems rather specific, the exponential form is in fact very general. The function allows changing the scale (parameters $d_1$ and $d_2$), the relative strength (parameter $b$) and the shape (parameters $h_1$ and $h_2$) of the spatial interaction kernel. Nevertheless, other functions, for example rational functions, could give similar results.

### 2.2. Cellular automata simulations of swarming

We apply the spatial kernel in a cellular automata model, called SIMSWARM, in which the nodes of a 2D orthogonal grid represent patches in an ecosystem (e.g. locations in a Petri dish). Patch colonization is calculated numerically using a 2D convolution operation with a convolution kernel (see also Rietkerk et al 2004). The simulations are initiated by seeding a small circular shape at the center of the grid, corresponding to the experimental setup. Repeated iterations of growth result in colony expansion. At the end of a simulation we can observe different shapes depending on the parameters $b$, $d_1$, $h_1$, $d_2$ and $h_2$.

Figure 3(A) shows a range of colony shapes produced by SIMSWARM. These particular simulations were carried out using high values for the exponents ($h_1 = h_2 = \infty$) effectively by implementing step functions for the positive and negative interactions. We use a shape factor, defined as the log 10 of circularity, to quantify the colony shape (see methods). This shape factor has a high value when the colony is round and a low value when the colony is branched. Under these conditions ($h_1 = h_2 = \infty$), low values of both $d_1/d_2$ and $b$ generate tiny colonies, which practically do not expand. Such shapes resemble colonies of immotile mutants of
Figure 3. Spatial kernel influences colony morphology, and there is a narrow range of parameters generating branched colonies. (A) Colony shapes produced using a cellular automata model (SIMSWARM) based on the iterative application of the spatial kernel. Parameter $b$ (the strength of the positive process) is varied across rows and parameter $d_1/d_2$ (the scale of the positive interaction) is varied across columns. The white color represents niches currently occupied by bacteria and the grey color represents niches that were at some point occupied by bacteria (the colony tracks). (B) Simulations reveal that for each value of $d_1/d_2$ branching occurs only in a narrow window of $b$. The colony shape is quantified as the $\log_{10}$ of the colony circularity. The black squares represent the array of parameter values used for the simulations in panel (B). All simulations were conducted with $h_1 = h_2 = \infty$. 
**P. aeruginosa** such as those of flagella-less mutants (Kohler et al 2000). Conversely, high values for both parameters means that the positive interaction dominates and the colony expands in a circular shape occupying the entire computational space. These shapes resemble colonies of hyperswarmers (figure 1(D), right panel), which have a typical shape factor of $-0.7$ as measured from experiments using image analysis (van Ditmarsch et al 2013). When the value of $d_1/d_2$ is fixed, there is a window of values of $b$ for which the shape is branched. This resembles colonies of the wild type **P. aeruginosa**, which have a typical shape factor of $-1.7$ (van Ditmarsch et al 2013).

The simulations suggest that, at least for the extreme values of $h_1 = h_2 = \infty$, colony branching is possible but only in narrow region of the parameter space. Plotting the circularity of colony shapes allows identifying this parameter region (figure 3(B)). The plot reveals a narrow diagonal band in parameter space where circularity is low, confirming that branching is only possible in this narrow combination of parameters.

### 2.3. Distance of positive interaction

The model presented above assumes that there is a length scale $d_1$ associated with dispersal. What is that length-scale in swarming colonies? Swarming requires production and secretion of large amounts of rhamnolipid biosurfactants by individual cells (Deziel et al 2003). These biosurfactants accumulate in a density-dependent way and lubricate the surface, thereby allowing the bacteria to disperse using active flagellar motility. Without surfactant secretion, however, a colony cannot swarm. Interestingly, a biosurfactant non-producer strain that is incapable of swarming regains its motility when mixed with a surfactant-producer by using the surfactants (Xavier et al 2011). Thus, biosurfactant secretion may be seen as a cooperative trait. Once secreted, surfactants will benefit all bacteria in the population including individuals that do not produce surfactants themselves (Xavier et al 2011, de Vargas Roditi et al 2013).

In order to assess the length-scale of surfactant-based dispersal we used two genetically altered strains of **P. aeruginosa**: a strain lacking biosurfactant production (a defector, strain PA14 $\Delta rhlA$), labeled with GFP (green fluorescent protein), and an inducible surfactant producer (a cooperator, strain PA14 $\Delta rhlA$ attB::P$_{BAD}$-$rhlAB$), labeled with DsRed-Express. The producer strain is induced to secrete surfactants constitutively by adding L-arabinose to the media (de Vargas Roditi et al 2013). We observed how the distance between the two colonies influenced the dispersal of the defector strain when both strains where placed on the same plate. Knowing the initial proportion ($p$) of defectors was 0.5, we measured the final proportion of defectors and calculated the change in defector proportion ($\Delta p$, figure 4(A)).

When both strains are mixed in the same seeding population (distance between cooperators and defectors is 0), the defectors increase in proportion over the course of the 24 h swarming assay ($\Delta p = 0.25$). The reason for this increase is that the defectors benefit from the surfactants produced by the cooperator strain but do not pay the associated metabolic cost (de Vargas Roditi et al 2013). However, when the colonies are seeded separately, $\Delta p$ decreases. Biosurfactants secreted by the producer have limited dispersal range. If the colonies are seeded too far away from each other only producers benefit and non-producers stay immotile. This is evident in pictures taken from the swarming plates (figure 4(B)). In those pictures the green defectors spread only when seeded together ($d = 0$) or at short distances from red producers. As the seeding distance increases, defectors receive less surfactant and thus are less capable of spreading. We reconstructed the change in defector proportion as a function of the distance to
Figure 4. Rhamnolipid secretion by a rhamnolipid-producing strain (cooperator) increases the dispersal of neighboring non-producer strain (defector) in a distance-dependent way. (A) The proportion of defector in the plate decreases with increasing distance to the rhamnolipid producer. Curve fitting was carried out by constraining the function $y = k(1)x / (k(2) + x) + m$ to cross the data point at $d = 0$ cm. The values obtained for the fit were $k(1) = -0.6996$, $k(2) = 0.2158$ and $m = 0.2443$; the critical distance at which the defectors receive only enough benefit to maintain the original proportion is 0.12 cm. Serial dilutions were used to start experiments from single-cells to increase the resolution of the assay. The data from those experiments is represented with an x. (B) Example pictures of swarming plates used to generate the data in panel (A). The producer strain is labeled in red and the defector strain is labeled in green.
itself also mediated by the surfactants (Caiazza et al 2005). They are both lubricants that facilitate colony spreading, as seen above, as well as long-range repulsion agents that prevent branches from crossing each other (Tremblay et al 2007).

We studied this process by seeding two surfactant-producing colonies (wild type P. aeruginosa) in the same plate and measuring the closest distance ever reached between the two colonies (figure 5(A)). We observed that the colonies could merge if they are seeded very close to each other (e.g. figure 5(B), d = 0.32 cm). However, as the colonies are seeded further apart the two colonies never merge and we observe repulsion (figure 5(B), d = 0.7, 1.96 and 4.37 cm). Next, we measured typical repulsion distances using a line inoculation, which allows swarming tendrils to develop parallel to the inoculation line as they spread away (figure 5(C), inset). A histogram of repulsion distances was constructed by measuring the distance between neighboring tendrils across a line parallel to the inoculation line at a distance of 1 cm (figure 5(C), main plot). The distribution reveals a median repulsion distance of 0.55 cm and a mode of 0.4 cm.

2.5. Simulations with realistic parameter values

Our experiments demonstrate the existence of counteracting processes that act at different length scales. Assuming that the distances measured for positive interaction and colony repulsion correspond to the distance parameters in our model (i.e. $d_1 = 0.12$ cm and $d_2 = 0.4$ cm) we used a parameter optimization routine to estimate the remaining parameters: the positive interaction strength, $b$, and the shape-defining exponent, $h$ (we assumed that both exponents $h_1$ and $h_2$ had equal value, and hence $h = h_1 = h_2$). We obtained the following expression:

$$f(d) = 11.8 \times 2^{- (d/0.12)^{0.3}} - 2^{- (d/0.4)^{0.3}}.$$  (5)
Figure 6. Simulations with a model of branching morphogenesis calibrated for *P. aeruginosa* swarming colonies. (A) Iteration steps from a simulation started from a spot inoculation (see also video 2, available from stacks.iop.org/NJP/16/015006/mmedia). (B) The optimized spatial kernel. Parameters $d_1$ and $d_2$ were constrained based on our experiments, whereas $b$ and $h$ were obtained using an optimization routine based on applying the interaction kernel to an actual image of a swarming colony. (C) Iteration steps from the simulation started from a line inoculation. (D) Pictures from colonies of *P. aeruginosa* started from a line inoculation.

Figure 6(A) shows a SIMSWARM simulation computed using equation (5) (see also video SV2). The shape obtained at the end of the simulation (see methods) has a shape factor of $-1.73$, which matches closely the typical shape factor of wild type *P. aeruginosa* of $-1.7$ (van Ditmarsch et al. 2013). The spatial kernel obtained from the optimization routine (figure 6(B)) depicts a broad but shallow neighborhood of inhibition and a narrow but tall region of enhancement. This is expected from our analysis of the model parameters (figure 3(B)), since a low value of $d_1/d_2$ requires a high value of $b$ to produce branching. We also used the parameterized SIMSWARM to simulate the line-inoculated experiments (figure 6(C)). Although these simulations are not meant to be an exhaustive test to our model, we did observe good qualitative agreement with the pictures of parallel tendrils obtained from line inoculations (figure 6(D)).

3. Conclusion

Our results demonstrate that a simple model implementing counteracting processes acting on different length-scales can indeed recreate branching patterns similar to those of swarming colonies. The kernel-based phenomenological model presented here draws from ecological theory, which has long recognized the relevance of distance-dependent processes as drivers for spatial patterning (Levin 1992). Many concepts from patterning in ecology are intimately related with the chemical basis of morphogenesis first proposed by Turing (1952), who first explained that counteracting positive and negative chemical processes acting on different length-scales can lead to symmetry-breaking that triggers biological patterning (Morelli et al. 2012). The model presented here is inspired by Turing’s findings but uses the spatial kernel approach of
recent population ecology models (e.g. Rietkerk et al 2004, Lindstrom et al 2011) rather than reaction–diffusion processes.

Which physical or biological components play a role in branching colonies of swarming *P. aeruginosa*? Our experiments support previous observations that rhamnolipid biosurfactants are crucial, since they have a dual role in swarming expansion: on the one hand, the surfactants are required to lubricate the surface, and can even enable motility of cells that do not produce rhamnolipids (figure 4(B)). On the other hand, the colony-repelling effects of surfactants (Caiazza et al 2005, Tremblay et al 2007) make colonies avoid each other (figure 5(B), supporting video 1, available from stacks.iop.org/NJP/16/015006/mmedia) and ensure that branches within a colony do not cross. The shape of the spatial kernel (figure 6(B)) may result from the combined effects of rhamnolipid synthesis, dispersal and their action on the motility behavior of individual bacterial cells.

Our model provides a coarse-grained approach by avoiding the explicit representation of such biophysical mechanisms. Nevertheless, more detailed models can provide valuable insights into swarming *P. aeruginosa*. For example, a recent multiscale model coupled a continuous fluid submodel and an off-lattice submodel for individual cells with high mechanistic detail (Du et al 2011). It is also noteworthy that the time-discrete integro-difference approach used here, just as in the ecological models on which our model is based (Rietkerk et al 2004, Lindstrom et al 2011), neglects differences in time-scale between the different processes such as cell growth and biosurfactant-based repulsion. These time-scales may be necessary to describe complex temporal patterns such as high-density waves observed recently in *P. aeruginosa* colonies using multispectral imaging (Du et al 2012). Other biophysical process that are not explicitly modeled here but that are known to play important roles in swarming include quorum sensing and metabolic prudence which control rhamnolipid production (Xavier et al 2011), surface tension (Du et al 2011, Fauvart et al 2012), spreading of liquid at the edge of a swarm (Tremblay et al 2007) and flagellated motion of bacteria in liquid (Kohler et al 2000, Du et al 2011, van Ditmarsch et al 2013).

In spite its simplified assumptions, kernel-based models illustrate how distance-dependent interactions can generate patterns of diverse shapes independently of specific mechanisms. These findings may be general beyond bacterial swarming. Even though mechanistically spatial interaction in other systems will be quite distinct from biosurfactant-mediated repulsion, the underlying concept of distance-dependent interactions can still hold. For example, in mammary glands TGF-β is secreted by cells, diffuses away from producing-cells and then triggers branching in a distance-dependent manner (Nelson et al 2006). Thus very distinct processes can lead to the similar types of spatial interactions.

A major difference between *P. aeruginosa* swarming and the development of multicellular tissues and organs is that swarming colonies do not constitute a multicellular organism, but are rather an assembly of unicellular organisms engaged in a collective trait. Here we focused on the branching patterns of monoclonal colonies, but genetic heterogeneity, which can originate for example from spontaneous mutation, is expected to change the outcome radically. In fact, the conflict between individual and group is known to be crucial to the ecology and pathogenesis of *P. aeruginosa* (Griffin et al 2004) which makes this bacterium a prime model for studying social evolution (Boyle et al 2013, de Vargas Roditi et al 2013).

Recent work from our group showed that repeated passaging of *P. aeruginosa* populations in swarming conditions leads to the evolution of hyperswarming mutants (van Ditmarsch et al 2013). The simulations conducted with SIMSWARM (figure 3) suggest that hyperswarmers
may achieve their advantage by altering the spatial kernel that governs their dispersal. By altering the interaction kernel, hyperswarming populations end up disrupting the branching process. It is compelling to make the analogy between hyperswarming and cancer, which occurs when mutation produces cell lineages that compete among each other and with the cells in the multicellular host (Merlo et al. 2006, Chen and Pienta 2011). Cell growth within the microenvironment of solid tumors is spatially confined, which can make spatial ecological interactions between neighboring cells critical (e.g. Gerlee and Anderson 2008, Orlando et al. 2013). Just like hyperswarmer mutants in a swarming colony, malignant cancer cells can be interpreted in light of our model as lineages with altered spatial interaction kernels that disrupt tissue organization. For example, the ability to evade growth suppressors, a hallmark of cancer (Hanahan and Weinberg 2011), would decrease the relative weight of the negative interaction of neighbors (i.e. cells in the vicinity secreting growth suppressors) leading to an increase in the relative strength $b$. Conversely, the activation of invasion and metastasis, another hallmark of cancer (Hanahan and Weinberg 2011), would increase dispersal, and therefore increase the ratio $d_1/d_2$. There is presently much interest in understanding how concepts from microbial populations, such as biofilms, apply to cancer and other multicellular systems (Lambert et al. 2011, Ben-Jacob et al. 2012). The same principles governing ecological interactions in spatially structured bacterial colonies may explain pattern disruptions observed in cancer.

4. Methods

4.1. Experimental measurements of distances of colony interaction

Two genetically altered strains of *P. aeruginosa* were used in this study: $\Delta rhlA\ attB::P_{BAD-rhlAB}$ (which produces biosurfactants when induced by $l$-arabinose) labeled with $attTn7::P_{A1/04/05}^{A1/04/05}$-dsRed Express and $\Delta rhlA\ attTn7::P_{A1/04/05}$-gfp (a green fluorescent clone that never produces rhamnolipids but is capable of utilizing the rhamnolipids produced by others) (Xavier et al. 2011, de Vargas Roditi et al. 2013). All bacteria were cultured in Lysogeny Broth Miller liquid media overnight. 1 ml of overnight starter cultures were washed twice with $1 \times$ PBS, and re-suspended in 1 ml of $1 \times$ PBS. Both strains were inoculated in 2 $\mu$l on standard swarming plates (de Vargas Roditi et al. 2013) complemented with 0.25% (w/v) $l$-arabinose. The inoculation distances between two strains were 0–5 cm. Plates were scanned after 24 h at 37°C with a fluorescence scanner. Cell mixes taken from each soft agar plate were serial diluted, inoculated on hard agar (1.5% (w/v)) plates, and scanned after 16 h at 37°C. Colony-forming units (CFUs) were counted as described elsewhere (Xavier et al. 2011).

The final proportion of defectors in total ($P_i$) was calculated from the CFU values and used to calculate the change in proportions using

$$\Delta P_i = P_i - P_0,$$

where $P_0$ is the original proportion of rhamnolipid producers ($P_0 = 0.5$). Distances ($D_i$) between cooperator and defector seeds on soft agar plates were measured using ImageJ (Schneider et al. 2012). Error bars represent the 95%-confidence intervals from estimation of binomial distribution fitting.
4.2. Repulsion distance

A green labeled wild-type (*P. aeruginosa*) PA14 *attTn7* :: *P* A1/04/03-gfp was used. An overnight starter culture was washed as previously described. Two seeds were inoculated on swarming plates at 0–5 cm distance. All plates were incubated at 37 °C for 24 h and then scanned. Distances between two seeds with corresponding minimal distances between the two populations from each plate were measured using ImageJ and plotted on a 2D plot.

4.3. Quantification of colony shape

Colony shape was determined using image analysis as follows: (i) grayscale images were segmented using direct thresholding; (ii) the area occupied by the colony (A) and the colony’s perimeter (P) were quantified using image analysis; (iii) colony circularity was calculated as 
\[
\left(\frac{4\pi A}{P^2}\right)
\]
and the colony shape was quantified as the log of the colony circularity.

4.4. Mathematical model

The mathematical model—SIMSWARM—was implemented in Matlab (the Mathworks, Natick MA) to simulate colony growth. The simulations’ environment implements a 2D computational space of 451 × 451 grid nodes each representing a 200 × 200 μm niche (corresponding to the resolution of a fluorescence scan of a swarm). The simulations are initialized by colonizing a round area at the center of the system representing an inoculum of 2 mm radius. White noise is added to the spot to break the symmetry. At each iteration of the simulation time, SIMSWARM uses the native Matlab 2D filtering function, *filter2*, to calculate the distance-dependent interactions between ecological niches. The convolution algorithm calculates colonization in the following time step at each generation. The edge of a plate (8 cm wide) is considered to exert a negative influence on colony growth to prevent colonies from reaching the edge of the plate. Simulations were stopped once colony expansion stops. We include a SIMSWARM implementation in Matlab code in the supporting material (available from stacks.iop.org/NJP/16/015006/mmedia).

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