

Diversity of evolutionary routes in range expansion

Context

Biological invasions and range expansion (population expansion into space that was previously unoccupied) can be substantially altered by evolution (Hallatschek et al, 2009). There is now a large body of experimental work demonstrating that range expansion favours evolution towards faster dispersal (Chuang et al, 2016). Most theoretical models consider cases where a mutant's advantage comes from an improved ability to reach the range margin, where resources are still plentiful, and competition for resources is the only interaction between a mutant and its ancestors. More nuanced scenarios are drawn from range expansion situations where direct interactions (mechanical or chemical interactions) are considered. Examples of such scenarios include slow-down of expansion and complete arrest of expansion (Korolev, 2015). But experimental support is still lacking. Here, we propose to explore the diversity of evolutionary routes in complex range expansion, by using a model system, swarming in the bacterium *Pseudomonas aeruginosa*.

Preliminary data

P. aeruginosa is a gram-negative bacterium that harbors one flagellum and can swim in water. It cannot move at the surface of an agar gel because of friction. However, when a colony reaches a certain size on the gel, it starts secreting surfactants (rhamnolipids) that spread on the surface, lower the surface tension, and allow the colony to expand dramatically. This behaviour is called swarming and generates striking branched-shape colonies. It requires motility and surfactant production. It is an example of range expansion where most of growth takes place near the edge, where the resources are still available.

In the past, we evolved and studied a hyperswarmer mutant that is able to cover the entire plate (van Ditmarsch et al, 2013; Deforet et al, 2014). This experimental evolution was performed with hard selection (the whole population was collected after each round and a constant ratio was transferred to a fresh plate). The hyperswarmer mutant is multi-flagellated; it is able to move near the colony edge and cut access to nutrients to its ancestor. This evolutionary pattern is in agreement with the literature about evolution towards better dispersal (Deforet et al, 2019). This mutant increases the area covered by the colony and also increases the total size of the population, hence it is more likely to be passed to the next plate.

More recently, we reproduced this experimental evolution and obtained the expected outcome: multi-flagellated, hyperswarmer mutants (see an illustrative replicate in Figure 1, upper row). Surprisingly, we also obtained mutants that do not spread at all (see an illustrative replicate in Figure 1, lower row). Preliminary data offer an explanation: Some non-spreading mutants lost the ability to secrete surfactants while others lost their flagella. Here we have a scenario where evolution leads to two completely opposite phenotypes: better spreading or worse spreading.

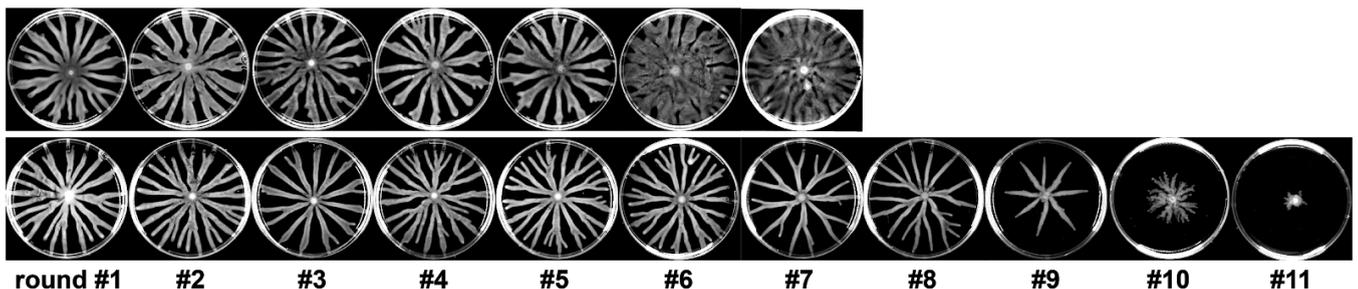


Figure 1 - Two independent replicates of experimental evolution. The upper replicate shows an increase of spreading in 7 rounds. The lower replicate shows a decrease of spreading in 11 rounds. Each plate is 10 cm in diameter.

This scenario raises evolutionary questions. Better coverage of the plate yields to greater population size in the case of the hyperswarmer mutation. How can a mutant that covers less area and form

smaller populations take over the ancestor? It also raises physics questions: in practice, what is the advantage of being non-motile? or of not producing surfactants?

We propose to combine evolutionary and physical approaches to decipher the mechanisms leading to the evolution of swarming behavior in opposite directions.

Proposed work

This project aims at identifying the mechanisms leading to the diversity of evolutionary trajectories, both from experimental and theoretical approaches. These two approaches will be coordinated and supervised by two PhD advisors.

Experimental approach:

- Preliminary experiments led to three outcomes: hyperswarmers, flagella defectors, surfactant defectors. We will explore the contingency scenarios and calculate the frequency of each outcome by replicating the evolutionary experiments at least 50 times, and by varying the experimental conditions (hardness of agar gel, addition of synthetic surfactants that could favor emergence of surfactant mutants)
- In the current protocol, cells inoculated at each subsequent round are randomly sampled from the whole previous plate. In subsequent experiments, we will vary the way the cells are sampled: for instance, we will sample cells from the colony edge only, or we will apply soft selection, not hard selection (a fixed number of cells are used for next round inoculation; Débarre & Gandon 2011).
- The effect of the three types of mutants on the competition needs to be quantitatively explored.
 - The hyperswarmer advantage comes from better dispersal that allows them to sit at the edge, where the resources are still available (see Figure 2).
 - Surfactant knockouts cannot swarm at all because of the friction against the gel. At low density, surfactant knockouts are free riders that take advantage of surfactants produced by the wild type, without paying the cost of producing surfactant. But at higher density of surfactant knockouts, surfactants are not sufficiently produced and the colony cannot spread at all.
 - Flagella knockouts cannot swarm. However, preliminary data shows they can “surf” the wave of surfactants secreted by the colony. This passive motility gives these non-motile the ability to precede the expanding front of motile cells, which remain at the center, and benefit from having better access to nutrients. If the non-motile cells are numerous enough, they can eventually block the expansion.

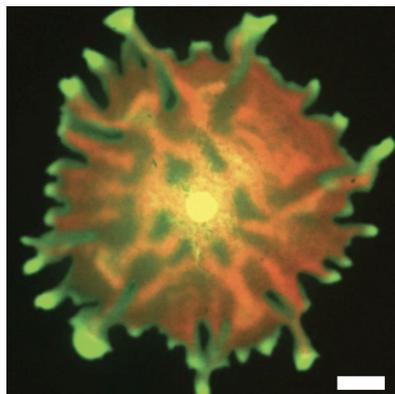


Figure 2 - Snapshot of the competition between wild-type *P. aeruginosa* (labeled in red), and its hyperswarmer mutant (label in green). Note how the hyperswarmers self-segregate near the colony edge, thanks to a better dispersal. Scale bar is 1 cm.

These preliminary results hint at a frequency dependence of the evolutionary advantage of each mutation. We will use fluorescently tagged strains to perform competition experiments with various initial ratios. Cells will be collected 24h later and counted, either by flow cytometry or CFU counting.

- To dig deeper into the mechanisms that bring advantages to each mutant, we will use a suite of imaging techniques to observe the spreading dynamics of monoclonal and mixed population colonies. We are equipped to perform live time-lapse imaging at 37°C across spatial scales. The imaging tools range from a whole plate imager down to an inverted microscope, capable of phase-contrast and fluorescence imaging.

Theoretical approach:

- We will develop mathematical models of the experiment as an attempt to identify the factors leading to one or the other evolutionary outcomes.
 - A first set of models will keep the metapopulation structure of the experiment, but will ignore spatial structure within each plate, and will look for conditions under which spiteful strategies like non-swarming can evolve;
 - A second set of models will concentrate on within-plate dynamics, and will aim to reproduce the spatial patterns of swarming and non-swarming types observed in the experimental data
- We will develop a second set of models representing different experimental protocols, and in particular with soft selection, i.e. with fixed numbers of cells taken from each plate before mixing, rather than mixing them all. We will develop predictions on how this different protocol may affect the results, before testing them experimentally.
- We will develop models to explore the contingency of evolutionary outcomes, in particular the effect of the order of mutations that affect swarming behavior. We will in particular explore what happens when multiple mutants coexist, and which one is more likely to take over the population.

Scientific environment

This proposed project will be carried upon the direction of two PhD advisors:

- Maxime Deforet is a researcher at the Jean Perrin Laboratory, a biophysics lab (Institut Biologie Paris Seine). He is an expert in swarming motility in *Pseudomonas aeruginosa*, an experimental model system for studies in evolutionary dynamics in expanding populations. Maxime Deforet does not have an HDR yet. Didier Chatenay, from the same lab, will serve as the official PhD advisor until Maxime defends his HDR.

- Florence Débarre is a CNRS researcher at the Institute of Ecology and Environmental Sciences (IEES-Paris). She develops theoretical models in evolutionary ecology, and is particularly interested in how spatial structure affects evolutionary processes. She defended her HDR in 2019 at Sorbonne Université.

Both PhD supervisors have been part of a working group on environmentally mediated interactions, which resulted in a synthesis article (Estralla et al. 2019), but they have not closely worked together yet, in spite of their overlapping interests. The supervision of this PhD would allow the development of a fruitful collaboration between the two labs, who are interested in similar questions but use complementary approaches.

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