

## Mechanisms for the initiation of multicellularity in bacterial biofilms

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Major transitions in evolution create the prerequisite features which allow natural selection to occur at a new level of organisation. Heredity, variation and reproduction must all be produced at a new higher level in order for a new evolutionary unit to arise. The origin of these features at new levels of organisation has been problematic for both theoretical biology and in artificial life models — but several partial theories exist. The necessary features may arise under the action of adaptive processes on existing units, and/or with the potential support of self-organisation of some kind. Limited mechanisms of heredity, potentially including ecological inheritance of constructed niches, may play an important role in bootstrapping the early stages of a transition to higher-level selection on new units. But, in short, theories for the precise routes by which new biological individuals might arise remain mostly speculative.

Our programme of theoretical modelling work has been focussing on simple individually-adaptable characters that may be involved in initiating higher-level units of selection. It is known that two key determinants of the efficacy of higher-level selection, between-group variation and heredity of group characters (e.g. the species composition of a group), are significantly affected by the modification of some simple parameters such as initial group size in an aggregation and/or the size of dispersal propagules. Our models have demonstrated that if simple features such as group structure parameters, group size and dispersal modes can be affected by characters that are under individual adaptive control then conditions that effect significant higher-level selection can be selected for despite individual self-interests. These features are simple enough that we can begin an empirical programme to investigate and manipulate the relevant variables.

In this talk we describe an ongoing experimental programme to investigate parameters affecting the levels of selection using bacterial biofilms. The majority of bacteria spend most of their life cycle in single- or multi-species biofilms, complex collective structures formed when bacteria attach to surfaces, and in this form they display an extraordinary repertoire of coordinated behaviours and interactions. Bacterial biofilms have numerous high-impact application areas including bio-engineering, bio-remediation and medicine where controlling the adaptation and co-adaptation of bacteria is vital. Reproduction in biofilms may be either via shearing off of groups of cells or by the production of individual motile cells. Despite this, such groups are also able to disaggregate into individual cells which reproduce in a planktonic phase. Accordingly, these organisms, in this case *Pseudomonas aeruginosa* (a common opportunistic pathogen problematic in cystic fibrosis), provide an excellent model system to address questions concerning the transition to multicellularity. They are fast-growing and experimentally tractable, allowing us to perform multi-generational evolutionary experiments over a relatively short timeframe, and they naturally exhibit physical characteristics, such as microcolony formation, that implement group structure. They possess individual characters, such as siderophore production, that can be knocked-out to produce clearly identifiable ‘cheats’ and ‘cooperators’. Crucially, there are easily identifiable individual characters, such as extra-cellular matrix production, that clearly affect the grouping parameters that are of interest, such as group size and propagule dispersal. We present our experimental methodology for manipulating these characters and thereby parameters that affect the strength of group selection; a vital first step in tackling the investigation of evolutionary transitions in real organisms.