**Universality in Clonal Phase Separation in Densely Packed Biofilms.** Peter J. Yunker[1] Eryn Bernardy[2], Luke McNally[3], Jacob Thomas[2], Jennifer Pentz[2], Arben Kalziqi[1], Sam Brown[2], Brian Hammer[2] and Will Ratcliff[2]. [1]School of Physics, Georgia Institute of Technology, [2]School of Biological Sciences, Georgia Institute of Technology, [3]School of Biological Sciences, University of Edinburgh.

**Introduction:** Microbes face persistent public goods dilemmas. While extracellular goods can benefit a population, they also are susceptible to cheaters. The formation of spatial structure, plays a key role in microbial cooperative metabolism, preventing cells producing costly extracellular goods from being exploited by non-producing cheats. Most mechanisms that drive spatial assortment[1] require free space for microbes to grow into. However, microbes often live in dense, well-mixed communities, wherein these established mechanisms are ineffective.

Antagonistic interactions play a large role in determining the structure of microbial communities. In particular, ~25% of gram negative bacteria utilize a Type VI Secretion System (T6SS), which directly injects toxic effector proteins into neighboring cells on contact.

Here, we examine how T6SS killing creates spatially structured populations by initiating 'Model A' coarsening – a process previously observed only in non-living systems[2].

**Summary of Work:** Two mutual predator strains of *Vibrio cholera* were grown together on an agar plate. As a control, defective killer strains, which are identical to the mutual predators but lack the ability to kill, were grown as well. Confocal microscopy revealed that the mutual predators form distinct, clonally separated domains; conversely, defective killers do not separate into clonal patches, but remain well-mixed.

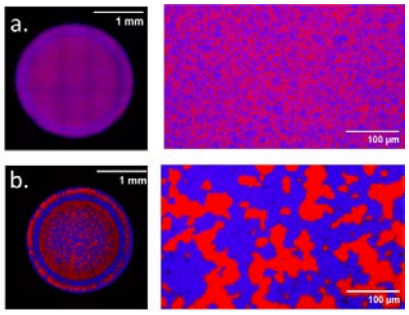


Figure 1. **"Model A" phase separation via T6SS contact killing.** a) Experiments performed with non-killing *V. cholerae* mutants show little phase separation in the colony (left) or zoomed-in view (right). b) Experiments performed with two mutual killing strains show "Model A" coarsening. In both (a) and (b), strains were initially well-mixed.

Agent based simulations, as well as PDE models replicate this behavior. Perhaps counter-intuitively, Ising-model simulations, developed to model magnetic materials, exhibit quantitatively identical dynamics. Analyses of these simulations and experiments reveal that clonal domains are formed via a 'Model A' transition. This transition is part of the broad non-conserved Ising universality class. While such transitions have been observed previously in alloys and many various abstract models, this observation demonstrates its biological relevance. This mechanism for structuring microbial populations is unique, rapidly creating genetic assortment even when the population is dense and population size is constant – conditions where established mechanisms for generating structure fail.

Consistent with our evolutionary predictions, we analyze 439 T6SS$^+$ bacterial genomes and find that genotypes containing more robust T6SS also possess larger secretomes, *i.e.*, more genes coding for extracellular products. In fact, after accounting for phylogenetic inheritance, variation in T6SSs explained the vast majority (90%) of variation in secretome size. The phase separation initiated by T6SS-mediated killing thus may thus be one of the most important factors enabling the evolution of cooperation in gram negative bacteria, with its effects visible in macro-evolutionary patterns of genome composition.

**Ongoing Work:** While we have experimentally shown that T6SS can drive spatial assortment, the universal nature of the 'Model A' transition suggests that many other approaches that feature local frequency selection may also rapidly produce spatial structure at high density. Preliminary simulations show that regardless of the details, local frequency selection processes produce spatial assortment from dense populations.

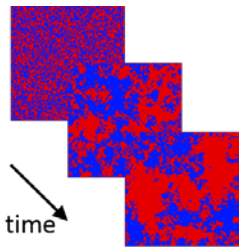


Figure 2. **Local frequency selection structures populations over time** Simulations of densely packed populations reveal that any mechanism that combines cell death with local frequency selection produces structured populations.

**References:** [1] Cates, M. E., Marenduzzo, D., Pagonabarraga, I. & Tailleur, J. Arrested phase separation in reproducing bacteria creates a generic route to pattern formation. *Proc Natl Acad Sci U S A* **107**, 11715-11720, doi:10.1073/pnas.1001994107 (2010) [2] Luke McNally, E. B., Jacob Thomas, Arben Kalziqi, Jennifer TPentz, Sam Brown, Brian Hammer, Peter Yunker, and William Ratcliff. *Nature Communications, In Press* (2017)