EMERGENCE OF COMPLEXITY IN CLONAL POPULATIONS EVOLVING UNDER CONSTANT RESOURCE LIMITATION. R. F. Rosenzweig[1,*], D. D. Yang[1], K. Schwartz[2], G. Sherlock[2], M. Kinnersley[3], K. Schmidt[3], P. Rashkov[4] and I. Gudelj[4]. *frank.rosenzweig@biology.gatech.edu; [1]Georgia Institute of Technology, 310 Ferst Dr., Atlanta, GA 30332; [2]Stanford University, 300 Pasteur Dr., Stanford, CA 94305; [3]University of Montana, 32 Campus Dr. Missoula, MT 59812; [4]University of Exeter, Stocker Rd., Exeter, EX4 4QD UK

Introduction: A microbial population that initially consists of a single clone can evolve into a population teeming with many, whether or not the environment is structured, and whether or not resource levels are constant or fluctuating.[1] Emergence of complexity, measured as functional information,[2] has been variously attributed to balancing selection, clonal interference and/or clonal reinforcement arising from either antagonistic or synergistic interactions among evolving lineages.[3] Using a combination of theory and experiment, we seek to define the boundary conditions under which one causal mechanism prevails over another.

Results and Discussion: Replicate populations of the bacterium *Escherichia coli* founded using the same ancestor and evolved under constant nutrient limitation differentiate into populations that are genetically complex, and in many cases, metabolically complex also. In the latter case, complexity can arise via specific cell-cell interactions such as cross-feeding.[4,5]

Cross-feeding clones isolated from one such experiment can be shown to increase each other's fitness, so that a consortium of co-evolved clones is not only more fit than their common ancestor, but also more fit than any single clone cultured by itself under the original evolutionary conditions.

We then developed an empirical model of cross-feeding based on bacterial energetics.[6] Our model indicates that the propensity for this type of metabolic complexity to evolve depends critically on resource input and population size, and perhaps also, specific mutations that unlock the capacity to scavenge scarce nutrients at the expense of redox balance.

To test these predictions we performed additional evolution experiments, founded by the same ancestor. We sequenced the resulting populations at very high coverage over hundreds of generations, and also carried out whole genome sequencing of scores of clones from each replicate. In none of these experiments did we uncover compelling evidence for synergistic interactions, but instead found massive clonal interference. This observation can be partly explained by the presence of myriad lineages often bearing mutations in the same gene, sometimes in the same location, leading to marginal differences in strain fitness. We also saw a plethora of mutations in different genes whose predicted effects would result in similar among-strain physiologies and fitnesses.

Taken together, our findings illuminate the various ways in which biocomplexity, viewed either in terms of ecological diversity or in terms of genetic diversity, increases under the simplest laboratory conditions we can devise: evolution of a single clone under constant resource limitation.

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