

Experimental evolution of bacterial restraint by selecting for growth yield

When microbes are cultured in an unstructured laboratory environment (e.g. a well-shaken flask) faster-growing mutants will typically arise and take over the population. There appears to be a fundamental metabolic trade-off in microbial species between rapid growth rate (r-selection) and efficient use of resources to maximize growth yield (K-selection). Takeover by a growth-rate strategy can be viewed as a "tragedy of the commons" where selfish variants that consume nutrients more quickly, but less efficiently, will be favored at the expense of decreased overall productivity in terms of cell yield. This laboratory result is seemingly at odds with the many cooperative and altruistic strategies observed in natural microbial populations, including costly siderophore and protease secretion, biofilm formation, and self-sacrifice.

In order to explore the evolution of cooperative behaviors, we will culture *E. coli* in the laboratory under conditions that favor variants with higher growth yields. This will be achieved by initiating thousands to tens of thousands of cultures in 384- or 1536- well microplates from single cells and allowing sufficient time for each to reach a saturating cell density. Mixing together these replicate cultures after growth, dilution, and redistribution of single cells into new wells for another growth cycle provides a pressure for cell yield because more efficient genotypes will colonize more wells at the next generation than less productive ones. A similar paradigm has been used to show that bacteriophage can be selected for burst size (i.e. yield per host) rather than replication speed in structured environments of bacterial hosts grown on agar surfaces.

As proof of principle we will use *E. coli* strains from a 20-year evolution experiment in well-shaken flasks that have twice the competitive fitness, but grow to only half the final cell density, of their ancestor. We will show that when many metapopulations are initiated from single cells there is competitive reversal such that the ancestor now realizes a greater fitness than the selfish evolved strains. Then, we will characterize what mutations give rise to the low-yield phenotype and select mutations that cause reversion to the high-yield phenotype of the ancestor from mutagenized libraries of evolved strains. Finally, we will enrich high-yield strains from *E. coli* single-gene knockout and overexpression libraries to probe the genetic plasticity of growth yield and the molecular mechanisms underlying this fundamental trade-off in bacteria.

Specific Aim 1. Determine what mutations cause reduced growth yield in selfish *E. coli* adapted to a well-shaken flask. Show that these strains are at a competitive disadvantage relative to their ancestors in replicate microwell plate cultures initiated from single cells.

Specific Aim 2. Enrich mutants of selfish *E. coli* strains with increased cell yield to determine to what extent and how they can revert to a restrained phenotype.

Specific Aim 3. Screen *E. coli* single-gene deletion and overexpression strain libraries for mutants with increased cell yield using microcultures initiated from single cells. Characterize the molecular mechanisms by which nutritional restraint is achieved.

The PI has relevant experience with *E. coli* evolution experiments and stochastic population genetic simulations that will be used to mathematically predict and interpret outcomes. Takeover of populations by selfish individuals that maximize yield of their own biomass rather than a desired bioproduct is a related problem in biotechnology. This work will provide an impetus for developing new technologies that enable millions to billions of microcultures to be initiated from single cells and screened individually for yield. With sufficient numbers of microcultures it may be possible to evolve restraint using only spontaneous mutations, to compare the evolved mechanisms to those found in nature, and to enrich cooperating communities of different genotypes by seeding cultures with multiple individuals at each generation.