# Seasonality gives rise to population oscillations in a bacterial cross-protection mutualism

Arolyn Conwill[1], Eugene A. Yurtsev[1], and Jeff Gore[1]

***Short Abstract* — Cooperative behavior plays a vital role in ecological communities and, when reciprocal, can result in mutualistic relationships. Here, we use an experimental microbial system to study the dynamics of a cross-protection mutualism in which two bacterial strains cooperate to survive in a multidrug environment. When subject to seasonal antibiotic dosing, the populations of the two mutualistic partners exhibit strong limit-cycle oscillations, even when there is long-term coexistence above the concentrations at which the individual strains can survive on their own. Our results provide insight into the ecological stability of mutualisms and the evolution of cooperative antibiotic resistance.**

***Keywords* — mutualism, seasonality, antibiotic resistance, oscillations, evolutionary dynamics, model system**

## I. Purpose

A mutualism arises between two species when each increases the fitness of its partner. Since mutualistic interactions between species are thought to be fundamental to the establishment and maintenance of ecological communities [1], much research has focused on studying mutualisms in their natural settings. Such studies suggest that the nature of the interaction between mutualistic partners is context dependent: while two species can be mutualistic in a harsh environment, they can nonetheless become direct competitors in a more benign environment [2]. Laboratory experiments with model systems (e.g., [3]), which are more readily controllable than their natural counterparts, can provide insight into the population and evolutionary dynamics that govern mutualistic relationships.

## II. Methods

Our model system consists of a bacterial mutualism that arises from cross-protection against antibiotics. Each strain produces an enzyme that deactivates one of the two antibiotics—ampicillin or chloramphenicol—thereby protecting cells from the second strain that would otherwise be sensitive to the drug [4]. Serial dilution experiments result in a seasonal environment: we dilute the bacterial population periodically by a fixed amount into new medium supplemented with the two antibiotics. The dilution strength, the time between dilutions, and the concentrations of the two antibiotics determine the strength of this periodic change in the environment. We experimentally track the population dynamics of the mutualism at the end of each growth cycle by measuring the density of each subpopulation using a combination of spectrophotometry and flow cytometry.

## III. Results

As the concentrations of the antibiotics in the environment increase, the two strains become increasingly dependent on each other for survival, with their interaction changing from primarily competitive to primarily mutualistic. Together the two strains can grow in antibiotic concentrations that inhibit growth of either one of the strains alone, thus forming an obligatory mutualism and enabling long-term coexistence.

In a seasonal environment (i.e. increased dilution strength, longer time between dilutions, and higher antibiotic concentrations), we observe strong limit-cycle oscillations between the subpopulations of the two mutualistic partners. Even so, the size of the total population exhibits little variation. In comparison, in the absence of seasonality (i.e. in the limit of a continuous environment), the population dynamics settle to an apparent equilibrium.

This obligatory mutualism persists until even stronger seasons give rise to oscillations so large that the population ultimately collapses. In particular, at one extreme of the oscillation, the population of one partner in the mutualism becomes so small that it can no longer protect its partner from the antibiotic it deactivates. Interestingly, often times the mutualism can successfully survive one dilution cycle before collapse, suggesting that the oscillatory dynamics cause the subpopulations to become too imbalanced to survive indefinitely in extreme environmental conditions.

We also investigate the evolutionary stability of the mutualism in the presence of potential invaders that employ different strategies, such as strains that are either sensitive or resistant to both antibiotics present in the environment. The outcomes provide insight about the evolutionary origins and resilience of such mutualisms.

## References

[1] Stachowicz JJ. (Mar. 2001) "Mutualism, Facilitation, and the Structure of Ecological Communities," *BioScience*, vol. 51, no. 3, pp. 235–246.

[2] Bronstein JL. (Jun. 1994) "Conditional outcomes in mutualistic interactions," *Trends Ecol. Evol.*, vol. 9, no. 6, pp. 214–217.

[3] Shou W, Ram S, and Vilar JMG. (Feb. 2007) "Synthetic cooperation in engineered yeast populations," *Proc. Natl. Acad. Sci.*, vol. 104, no. 6, pp. 1877–1882.

[4] Yurtsev EA et al. (Jan. 2013) "Bacterial cheating drives the population dynamics of cooperative antibiotic resistance plasmids," *Mol. Syst. Biol.*, vol. 9, no. 1.

Acknowledgements: This work was funded by National Institutes of Health R01 Grant No. GM102311-01. E.Y. was supported by the National Science Foundation Graduate Research Fellowship under Grant No. 0645960

[1]Department of Physics, Massachusetts Institute of Technology, Cambridge, MA. E-mail: gore@mit.edu