PHYSICAL CONSTRAINTS AFFORD EVOLUTIONARY OPPORTUNITY DURING THE TRANSITION TO MULTICELLULARITY. William C. Ratcliff. Biology Dept., Georgia Institute of Technology. 310 Ferst Dr., Atlanta, GA 30332, will.ratcliff@biology.gatech.edu.

The evolution of multicellularity was a major transition in biology, allowing for the origin of large, complex organisms. While multicellularity has evolved repeatedly in diverse lineages, crucial early steps in this transition (such as how simple clusers of cells evolve to be more complex) remain poorly understood, largely because the known multicellular lineages on Earth are ancient. Experimental evolution has emerged as a promising approach to bridge this knowledge gap because it allows researchers to directly observe the first stages in an evolutionary transition.

Using the unicellular baker's yeast, *Saccharomyces cerevisiae*, we have evolved simple multicellular clusters by selecting for rapid settling through liquid media. Over ~1,500 generations of continued selection, these nascent yeast clusters continued to adapt, mainly evolving to settle faster (Figure 1).

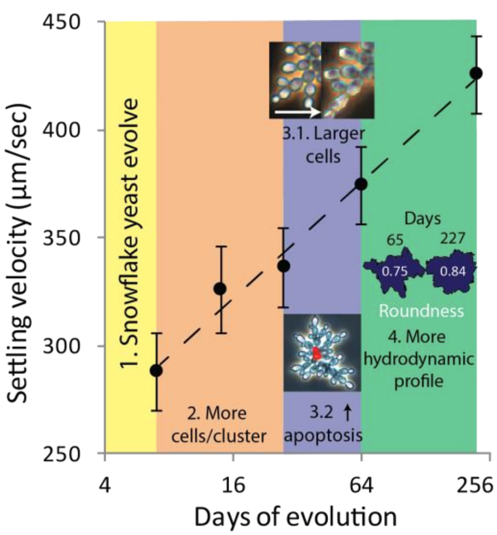

Figure 1. Multicellular adaptation in snowflake yeast. Modified from Ratcliff and Travisano. *BioScience* (2014). 64 (5):383-393.

Snowflake yeast accomplished this by evolving to form cluster containing more cells, increased cell size, and a more hydrodynamic cluster profile (Figure 1). Another trait, elevated apoptosis, appears to act differently. Large clusters settle quickly, but also grow slowly, probably due to diffusional limitation. Apoptosis results in cell-cell scission and the production of a greater number of smaller, faster-growing propagules, increasing fitness by overcoming this growth constraint.

In this talk, I will focus on how two physical constraints experienced by snowflake yeast underlie their remarkable rate of multicellular adaptation. The first, poor resource availability to internal cells due to diffusional limitation, creates an incentive for the evolution of increased complexity. Snowflake yeast experience two strong, and contrasting selective forces: every 24h they must be able to settle quickly, or they perish, but they must also compete for finite resources in the culture media in the intervening time. Diffusional limitation thus prevents the evolution of massive balls of undifferentiated cells that settle very rapidly, because the growth costs more than exceed the benefit of faster settling. Some of the adaptations that increase the complexity of our clusters (*e.g.*, elevated apoptosis, more hydrodynamic clusters) arise precisely because of this trade-off.

The second constraint I examine concerns the physical structure of the cluster. Snowflake yeast clusters form through the loss of a single gene (*ACE2*), and result from daughter-cell adhesion after mitosis. This extremely simple mode of cluster growth turns out to have important down-stream implications for multicellular evolvability. By forming branched-chains of cells that separate by scission, clusters exhibit a multicellular life cycle that includes single cell genetic bottlenecks and clonal development (Figure 2). This facilitates the evolution of multicellular-level traits by limiting the potential for genetic conflict among cells within the cluster, increasing the heritability of multicellular traits, and exposing the cluster-level effects of every *de novo* mutation to selection. Thus, a simple constraint in the way that cells attach provides the basis upon which the ability to evolve as a multicellular individual is founded.

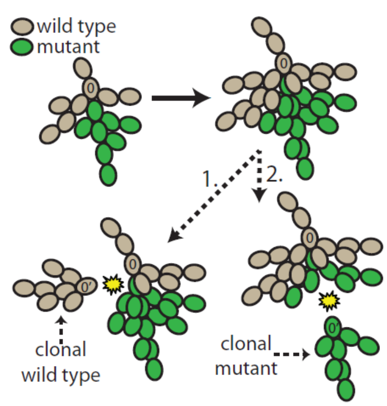

Figure 2. Bottlenecks caused by propagule separation (yellow bursts) purge within-cluster genetic diversity. Modified from Ratcliff et al., *Nature Communications* (2015). 6: 6102.