**EVOLVING MECHANICS OF NASCENT MULTICELLULAR SNOWFLAKE YEAST.** Shane Jacobeen[1] Jennifer Pentz[2], Elyes Graba[1], Colin Brandys[1], Will Ratcliff[2], and Peter Yunker[1]. [1]School of Physics, Georgia Institute of Technology, [2]School of Biology, Georgia Institute of Technology.

**Introduction:** The evolution of multicellularity was transformational for life on earth [1]. Though nascent multicellularity conveys a host of benefits, these benefits come at a price: groups composed of multiple cells must contend with previously irrelevant forces (internal and external) capable of breaking intercellular bonds. Therefore nascent multicellular groups must adapt to mitigate these fracture-inducing forces. To study the relationship between physical constraints and the evolution of multicellularity, we employ the snowflake yeast model system. It has been demonstrated that under daily selection for large size, the unicellular baker's yeast S. cerevisae develops a mutation that results in the formation of clonal clusters [2]. These clusters, called 'snowflakes' due to their fractal-like structure, evolve increased in size under sustained size selection. By studying the mechanical underpinnings that facilitate the nearly twofold increase in snowflake radius that occurs over 8 weeks of evolution (~330 generations), we are exploring the role of physics in the evolution of multicellular complexity.

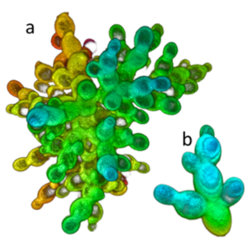


Figure 1. Large (a) and small (b) snowflake clusters. Note: different magnifications

**Summary of Work:** Using brightfield and atomic force microscopy, we determined that snowflake clusters fracture due to the accumulation of internal stress. This stress is the result of cells being displaced from their initial position by the addition of new cells. As a the number of cells in a cluster increases, the available free volume in which to add new cells decreases, so cells press against their neighbors with increasing frequency and force. When this force reaches a critical threshold set by the strength of the intercellular chitin bonds, a bond fails and the cluster fractures. As fracturing limits cluster size, the evolution of larger snowflakes is contingent upon mitigating the accumulation of internal stress caused by growth.

A simple cell-level change appears to play an essential role in the evolution of increased size in snowflake yeast. Though all *s. cerevisae* cells are slightly ellipsoidal, the cells of larger clusters that have undergone 8 weeks of selection are more elongated than the cells in the smaller clusters that first appeared after 1 week. Inspired by the fact that the increasing particle anisotropy can increase packing efficiency in non-living systems [3], we created a simulation to test the effect of cellular packing on stress accumulation in the snowflake growth form. Indeed, the increased packing efficiency resulting from cellular elongation resulted in a significant reduction in the rate of internal stress accumulation.

This increasing packing efficiency also increases the number of accessible non-fracture-inducing future growth plans. As snowflake yeast can fracture at any time during growth, it is beneficial for them to maximize the possible number of configurations at every step, a process related to causal entropy maximization [4]. Our simulations reveal that for similarly sized clusters, the energy stored in week 8 snowflakes is approximately half that of their week 1 counterparts; as clusters fracture due to the critical accumulation of stress, this indicates that week 8 clusters can add significantly more cells before their stress reaches the fracture threshold. Thus by increasing their cellular aspect ratio, snowflakes evolve to increase their size by mitigating the accumulation of fracture-inducing internal stress.

**Ongoing Work:** We have already demonstrated that packing efficiency and fracture mechanics play critical roles in the fitness of free growing multicellular clusters. Yet nascent multicellular organisms on earth did not live in isolation; rather they surely encountered external forces imposed by their environment. Therefore, my current endeavor is to test the performance of snowflake yeast evolving in the presence of external physical challenges. As an initial step, snowflakes were again evolved under daily size selection, however this time their ability to grow large was hindered by compression prior to selection. Preliminary results show that compared to the control strain, these compression-evolved snowflakes developed resiliency to compression. Though yet unknown, the mechanism of this resiliency will be the subject of our research in the coming months.

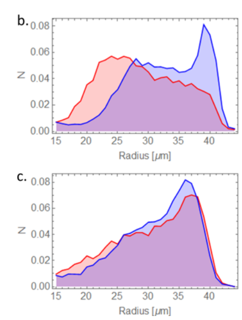


Figure 1. **Evolution with compression produces tough clusters** Size histograms for before (blue) and after (red) compression of control (a) and compression evolved (b) strains.

**References:** [1] Szarthmary, E. and Smith, J. M. (1995) *Nature, 90,* 227-232. [2] Ratcliff, W. C. et. al. (2012) *PNAS* 109, 1595-1600 [3] Donev et. al. (2004) *Science* 303, 990-993 [4] Wissner-Gross, A. D. and Freer, C. E. (2013) *Phys Rev Lett., 110.*