CLONAL DEVELOPMENT IS EVOLUTIONARILY SUPERIOR TO AGGREGATION IN WILD-COLLECTED *SACCHAROMYCES CEREVISIAE*. Jennifer T. Pentz[1] , Adaugo Asouzu[2], Michael Travisano[3], and William C. Ratcliff[4]. [1]Georgia Institute of Technology, 310 Ferst Dr., Atlanta, GA 30332, jennifer.pentz@gatech.edu. [2]University of Minnesota, Minneapolis, MN 55108, USA. [3]University of Minnesota, Minneapolis, MN 55108, USA, travisan@umn.edu. [4]Georgia Institute of Technology, 310 Ferst Dr., Atlanta, GA 30332, will.ratcliff@biology.gatech.edu.

Abstract: The evolution of complex life on Earth has occurred through several key steps in which formerly autonomous organisms evolve to become integral parts of a larger, higher-level organism. Maynard-Smith and Szathmáry termed these the major transitions in evolution[1], and a profound example of this is the transition from uni- to multi-cellularity, allowing for subsequent increases in biological complexity. There are two basic modes of multicellular body formation: clonal development via 'staying together' of mitotically-produced cells or potentially unrelated cells can 'come together' via aggregation. Virtually all extant multicellular taxa develop via 'staying together', with only a few species of microorganisms developing by 'coming together', suggesting that 'staying together' may be evolutionarily superior to 'coming together'. Theory predicts that 'staying together' should provide several key advantages over cells 'coming together' because this mode of development creates clonal clusters with little among-cell genetic variation, limiting the potential for evolutionary conflict, as there is little standing within-cluster genetic variation for selection to act on[2,3]. Second, any variation that arises due to mutation gets partitioned among multicellular offspring, allowing selection to act on the cluster-level effects of *de novo* mutation, increasing the scope for cluster-level adaptation. This also limits the potential for genetic conflict by preventing the proliferation of 'cheating' cellular lineages[2]. Despite these predictions, little empirical work has been done to directly compare these two developmental modes.

Previously, we have shown that selection for rapid settling through liquid media in the yeast *Saccharomyces cerevisiae* results in the evolution of cellular clusters that develop exclusively through post-division adhesion of mitotically-produced cells[4], although yeast are also capable of forming clusters by 'coming together' via adhesive glycoprotien production, termed flocculation[5]. This suggests that 'staying together' is superior to 'coming together' in this yeast model system, but this trait may just simply evolve more readily that flocculation. To test this latter hypothesis, we repeated the experiment performed by Ratcliff et al., 2012, starting with five wild-isolated highly-flocculant strains of *S. cerevisiae*. This effectively 'stacks the deck' in favor of aggregation, as all yeast start out with the ability to form multicellular clusters via flocculation.

After 155 daily transfers, snowflake yeast evolved and displaced their floc ancestor in 36 out of 40 replicate populations. Consistent with prior theoretical predictions, we find that early snowflake yeast (invading from rare) possess a striking fitness advantage over their floc counterparts[6]. In addition to any long-term evolutionary benefits provided by clonal development, mathematical modeling suggests that the snowflake yeast growth form exhibits less non-heritable phenotypic variation than flocculation[7]. Reduced developmental noise may provide a proximate benefit in our experiments, allowing snowflake yeast to 'tune' their multicellular phenotype to selective conditions, wasting less biomass on intermediate size clusters that do not survive settling selection. Further experiments are currently underway to disentangle the contribution of proximate and ultimate evolutionary benefits to the competitive superiority of snowflake yeast under our experimental conditions.

References: [1]Maynard Smith and Szathmáry. (1994) *Oxford Univ. Press* [2]Grosberg and Strathmann. (2007) *Annu. Rev. Ecol. Evol. Syst.* [3]Michod and Roze. (2001) *Heredity*. [4]Ratcliff *et al.* 2012. *PNAS*. [5]Smukalla *et al*. (2008) *Cell*. [6]Pentz *et al*. 2014. *ALIFE 14*. Ratcliff *et al.* 2015. *Nature Communications 6*.