Genetic Basis Underlying *De Novo* Origins of Multicellularity in Response to Predation. Kimberly Chen, Frank Rosenzweig and Matthew Herron. Georgia Institute of Technology, Atlanta, GA 30332 (kichen.evol@gmail.com).

Background: The evolution of multicellularity is a Major Transition that sets the stage for subsequent increases in biological complexity [1]. However, the genetic mechanisms underlying this major transition remain poorly understood. The volvocine algae serve as a key model system to study such evolutionary transitions, as this group of algae contains unicellular and fully differentiated multicellular species, as well as diverse extant species with intermediate complexity. Comparative approaches using the three sequenced volvocine genomes of unicellular *Chlamydomonas reinhardtii*, colonial *Gonium pectorale* and fully differentiated *Volvox carteri* have revealed important protein families for the evolution of multicellularity [2, 3, 4]. Nevertheless, this retrospective approach limits insights into the tempo and mode of genetic changes and the possible pathways a unicellular organism can explore for the transition to multicellularity. Experimental evolution in the laboratory allows direct observations of such transitions in real time.

We used the unicellular *C. reinhardtii* to generate *de novo* origins of multicellularity under predation. Outcrossed populations of *C. reinhardtii* were subjected to selection by the filter-feeding predator *Paramecium tetraurelia*. After 50 weekly transfers, two of five experimental populations evolved multicellular structures not observed in any of the three unselected control populations. Furthermore, the evolved isolates form multicellular structures via intraorganismal division similar to the volvocine algae such as *Eudorina*.

Methods: To uncover genetics underlying multicellularity, we isolated DNA from 24 isolates from the two experimental populations and one control population (8 from each population) and conducted Illumina whole-genome paired-end sequencing to identify genetic changes occurred in those evolved isolates. To investigate epistatic interactions among evolved mutations or with the ancestral genotypes, we conducted bulked segregant analysis (BSA). Evolved isolates were crossed with unicellular ancestors, and the resulting recombinant F2 progeny were sorted into multicellular and unicellular pools. DNA from each pool was sequenced to detect differences in allele frequency, and over-represented alleles in the multicellular pool are likely interacting alleles that contribute to the multicellular phenotypes.

Results: The multicellular isolates from the two experimental populations exhibit distinct genetic signatures from each other. The isolates from different populations were derived from different recombinant cells in the starting outcrossed population based on the patterning of the ancestral variants across chromosomes from the two parental strains. Within each population, the multicellular isolates share a number of mutations together, but not with the multicellular isolates from the other experimental population, or with the unicellular isolates from the control population. Each isolate also accumulated mutations not found in other isolates. In addition, the results from BSA suggest instances of epistatic interactions among ancestral variants and derived mutations, and we are currently working on elucidating such interactions underlying the origins of multicellularity.

Conclusions: We have identified the mutations that arose in the multicellular isolates from the two experimental populations and the possible genetic interactions linking to the emergence of multicellularity. Further, the modes of genetic changes underlying the multicellular phenotypes in the two populations appear to be distinct from each other. This is the first step towards understanding the dynamics and mechanistic basis of the evolution of complexity.

References: [1] Maynard Smith, J. and Szathmáry E. (1995) *Oxford University Press*. [2] Merchant, S. S. et al. (2007) *Science*, 318, 245-250. [3] Prochnik, S. E. et al. (2010) *Science*, 329, 223-226. [4] Hanschen, E. R. et al. (2016) *Nat. Commun.*, 7, 11370.