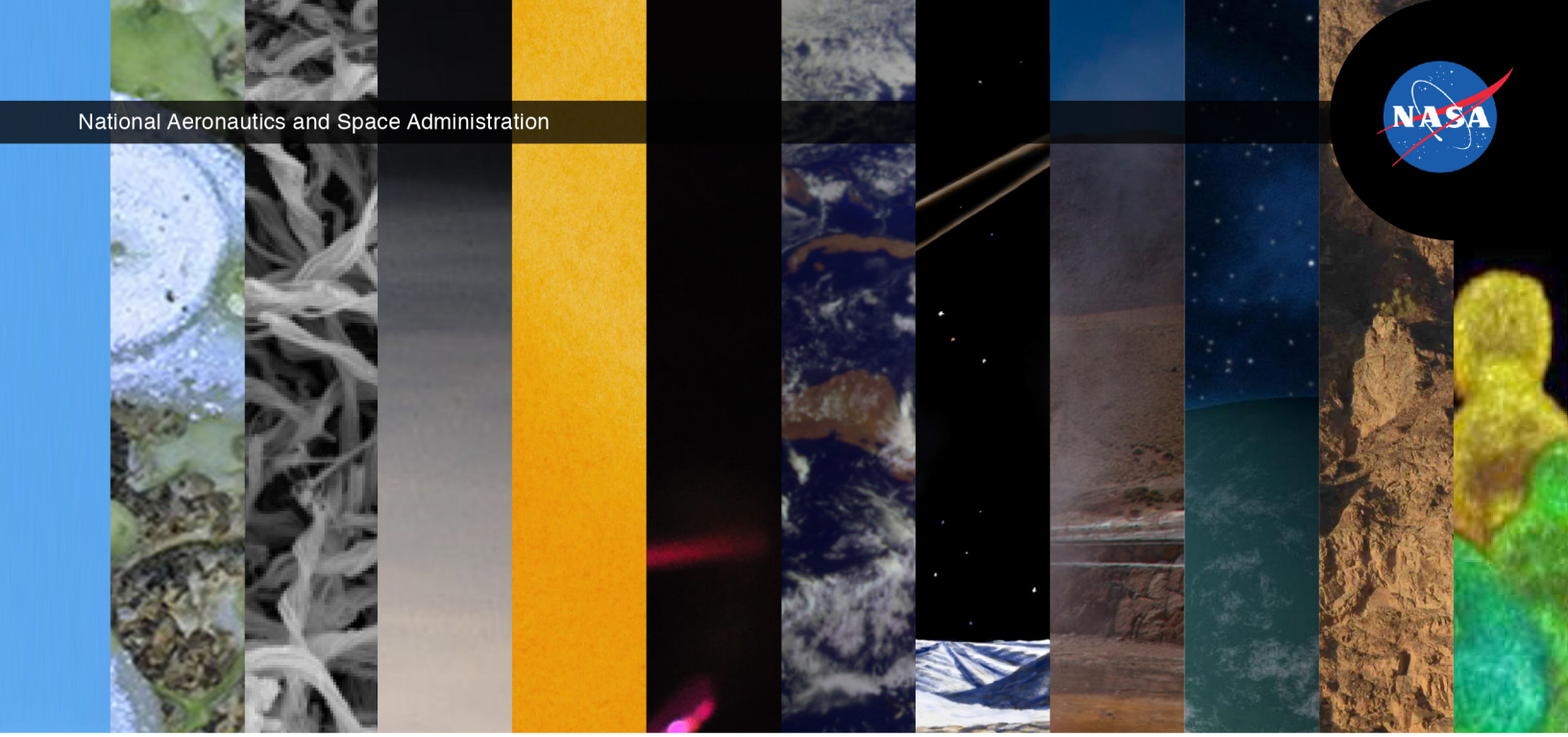
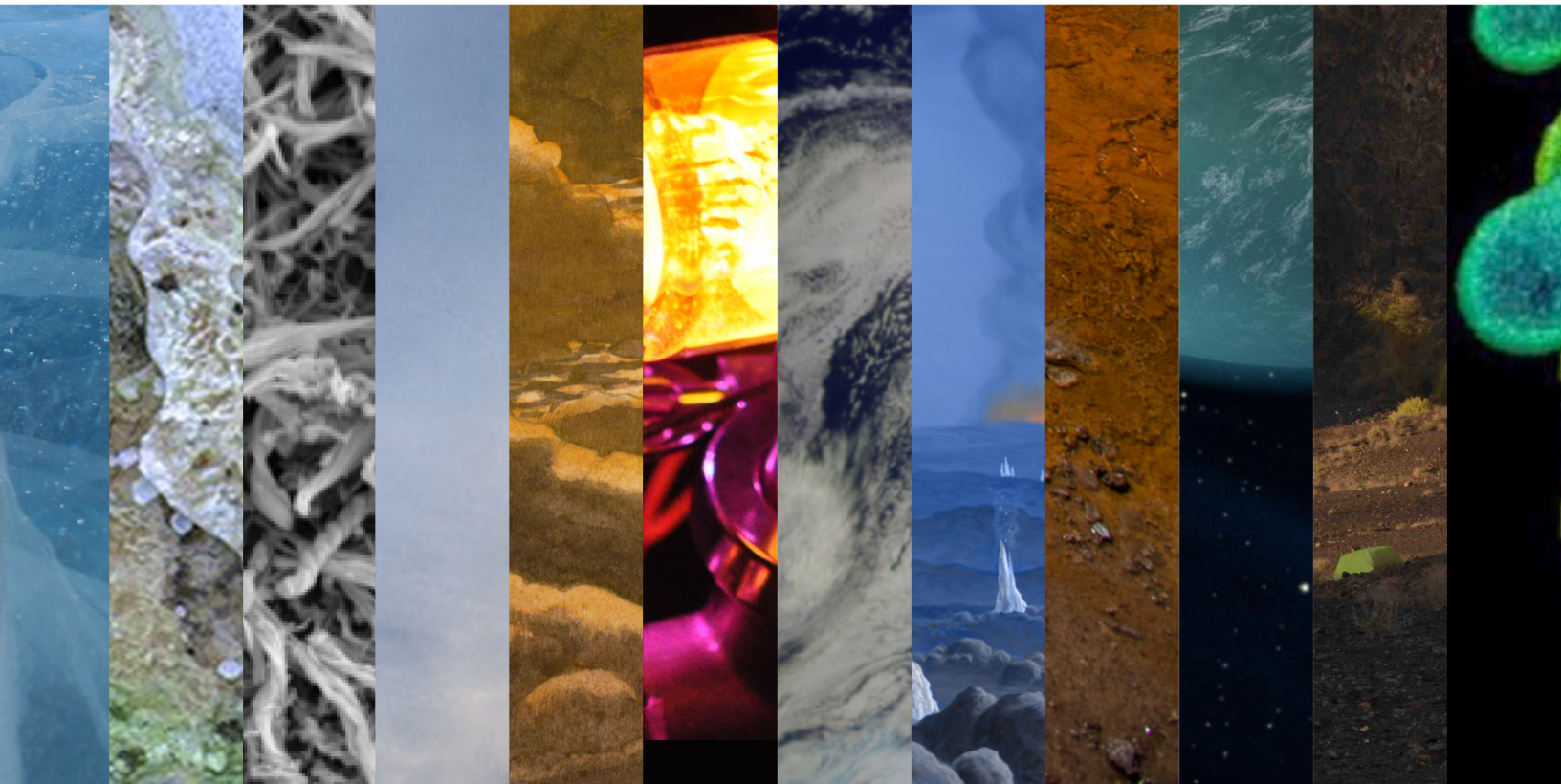


NASA Astrobiology Institute

2017 Annual Science Report

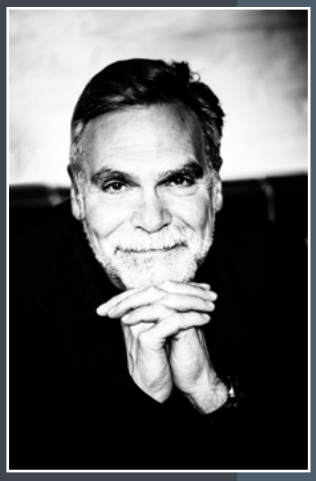
Reliving the Past: Experimental Evolution of Major Transitions

Georgia Institute of Technology



Reliving the Past:
Experimental Evolution of Major Transitions

Lead Institution:
Georgia Institute of Technology



Principal Investigator:
Frank Rosenzweig

Team Overview

The Origin of Species concludes with a hymn to biocomplexity teeming on an English hillside. The hymn's most penetrating verse is "these elaborately constructed forms, so different from each other, and dependent upon each other, had all been produced by laws acting around us." To delve into these laws, to understand how differences are selected and how interdependence is enforced remains biology's grandest challenge. Our Team seeks to meet this challenge by Reliving the Past using experimental evolution, an approach that enables us to discern evolution's causes as well as its consequences, and to discover why evolution takes certain paths and not others. By tackling five questions we aim to illuminate what drove major transitions leading to the evolution of complex life:

- How do enzymes and metabolic networks evolve?
- How did the eukaryotic cell come to be?
- How do symbioses arise?
- How does multicellularity evolve?
- How do history, gene interactions and mutation constrain innovation?

We seek general principles likely to govern the emergence of complexity wherever life exists. Our enterprise falls squarely within Astrobiology, the study of the origins, evolution, distribution, and future of life in the universe, and addresses the fundamental question: How does life begin and evolve?

2017 Executive Summary

Darwin's view of Nature was one in which the Struggle for Life figured preeminently, and, indeed, competition continues to be the conceptual framework in which we most often view natural selection. But cooperative interactions can also be (selectively) favored, and these are fundamental to all levels of biological organization, from enzymes to organelles, cells and societies of cells to organisms, and populations to communities. Our team is pursuing five questions related to major evolutionary transitions during the history of life on Earth. These transitions occurred when simple subunits coalesced to form autonomous, interdependent wholes, producing quantum leaps in biocomplexity. These overarching questions are: *How do enzymes and metabolic networks evolve? How did the eukaryotic cell come to be? How do symbioses arise? How does multicellularity evolve?* and *How do pleiotropy, epistasis and mutation rate constrain the evolution of novel traits?* If Life is a self-sustaining chemical system capable of Darwinian evolution, then the answers to these questions should apply wherever life exists. Our research enterprise thus falls squarely within the purview of Astrobiology, which is the study of the origins, evolution, distribution, and future of life in the universe, and helps to address Astrobiology's most fundamental question: How does life begin and evolve?

By 6 key performance indicators, the Reliving the Past Team enjoyed a highly successful Year-3. In terms of scholarly output we published, or now have in press, 47 peer-reviewed papers, including reports in high-impact journals such as *Nature Reviews, Nature Ecology Evolution, Nature Communications, Nature Physics*, and *PNAS*. Several papers attracted media coverage. Will Ratcliff's work was featured in New Scientist as well as on Norwegian National TV. Several team members have been promoted to leadership positions in their respective communities. Vaughn Cooper was elected Vice-Chair of the Gordon Research Conference on Microbial Population Biology; Betul Kacar accepted a faculty position at UArizona and is charged with spearheading their Astrobiology program; Paul Sniegowski was appointed Dean of the College of Arts and Sciences at the UPenn. Our team continues to build on the foundation of the NAI cooperative agreement: collectively, we submitted 19 new grant proposals. Many of these are still pending, but to date 9 new awards have been made totaling $5.9M. In 2017 "Reliving the Past" team members gave 60 invited seminars, and engaged in a broad range of synergistic activities. We provided peer review on scores of manuscripts, served on editorial boards for *BMC Microbiology, J Biological Chemistry, Genome Biology Evolution, Microbial Cell*, and *Biology Letters*, and were panelists for NASA, NSF and NIH. Team members provided mentorship to over 50 students ranging from high school trainees and undergraduates, to pre- and post-docs; this number includes three NPP fellows (Stephanie Weldon, Caroline Turner and Peter Conlin), one DDF Fellow (Kennda Lynch) and one NSF Pre-doctoral fellow (Jordan Gulli). The team's broader impacts were felt in a variety of education/public outreach activities that ranged from giving televised lectures to high school students, providing in-classroom and in-lab mentorship to high school teachers and students, to offering public, streamed lectures/interviews on the Origin of Life. Co-I Sniegowski's Princeton University Press volume, *Evolution of the Mutation Rate*, is slated for publication in 2018. PI Rosenzweig organized our September 2017 team meeting as the centerpiece of a widely-attended, 2-day public symposium: "Life in the Cosmos: A Celebration of Astrobiology Research." Invited speakers included colleagues Nick Hud and Loren Williams from the Center for Chemical Evolution, Jen Glass and James Wray, co-Is of the UC Riverside and SETI NAI teams, respectively, Scripps Professor Ram Krishnamurthy and "Icy Worlds" NAI Team PI, Isik Kanik. PI Rosenzweig also served as Senior Member of the Center for Theological Inquiry at Princeton, where the Center is engaged in a 2-year NASA-funded study on the Societal Implications of Astrobiology.

Our team has made substantial research progress towards goals articulated in our original proposal, and is also pursuing new opportunities. Below are but four examples. In one project (Consequences of *recA* duplication for recombination, genome stability and fitness), Scott Miller and his group at the University of Montana have made great strides toward understanding the evolutionary impact of possessing multiple duplicated copies of the recombination gene *recA* in the genome of the *cyanobacterium Acaryochloris*. Remarkably, the different copies have become differentially regulated in response to different environmental stressors and have also diverged in function. Scott also published a meta-analysis appraising the evidence for the widely-assumed trade-off between enzyme stability and activity (Miller, S. *Evolution* 71:1876), and this analysis has broad implications for our understanding of the biophysical constraints on enzyme adaptation to extreme environments. For example, adaptation to higher temperature often comes without a cost in performance at ancestral lower temperatures, thereby easing the path to adaptation to novel environments. Dr. Miller also launched a new project to characterize the physiology and genomics of a poorly-studied endosymbiosis between a diatom host and a cyanobacterium, which is in the process of becoming a nitrogen-fixing organelle.

Matt Herron and Will Ratcliff at Georgia Tech are investigating The evolution of multicellularity and cellular differentiation in Bakers yeast and in the Volvocine green algae. Between them they have at least a dozen interesting projects underway, including studies on the origin of multicellular development, evolution of multicellularity in response to predation, the role of oxygen in catalyzing / constraining the evolution of multicellular complexity, and on the materials properties of nascent multicellular organisms. With GaTech physicist Peter Yunker, Will has examined the physical basis for the evolution of larger body size in multicellular snowflake yeast (Jacobeen et al. *Nature Physics* 14: 286). Simple undifferentiated groups making the first steps in the transition to multicellularity need to evolve robust bodies capable of surviving forces that act over multicellular length scales. Through experiments and mathematical modeling, the team showed that yeast evolve to form more robust bodies by reducing the packing fraction of cells within the cluster (which reduced the strain placed on cell-cell connections). This was achieved by evolving a more elongated cell body. This work helps us understand how nascent multicellular organisms evolve novel multicellular materials properties. Will and Matt jointly communicated a paper to a special issue of *Phil Trans Royal Society, Series B* entitled "Process and pattern in innovations from cells to societies." Their study used mathematical modeling to describe how nascent multicellular life cycles play a key role in facilitating transitions in individuality.

Paul Sniegowski (UPenn) and Phil Gerrish (Georgia Tech) continue Exploring the relationship between mutation, recombination and cooperation. Two of their NAI supported students Tanya Singh (PhD 2016) and Mitra Eghbal (PhD 2017) published work this past year on the implications of deleterious mutations for the fate of evolving populations. One, an empirical paper, explored the relationship between mutation rate evolution and population persistence in small *Escherichia coli* populations (Singh et al. 2017. *Biol Lett* 13: 20160849); another, a theoretical paper, analyzed the process whereby lineages carrying beneficial mutations become progressively contaminated with deleterious mutations (Pénisson S et al. 2017. *Genetics* 205: 1305). Mitra explored factors affecting instability of mutation rates in asexual experimental populations of *E. coli*, demonstrating that mutator populations under repeated lethal selection can evolve even-higher mutation rates (Eghbal et al., in revision). Her finding provides further support for a main motivating idea behind Paul and Phil's work, namely that error rates in adapting asexual populations have an intrinsic upward instability that can culminate in extinction.

Eric Smith (Georgia Tech/Earth Life Science Institute) and Betul Kacar (Arizona/ELSI) seek to understand The role of information carried by relations in enabling major transitions. The relations may be among genes in a chromosome or gene pool, community members in a co-evolving population, or key enzymes within complex molecular systems. Smith's work addresses limitations in the abstraction of the replicator as a foundation for evolutionary genetics, a concept too narrow to encompass patterns interrupted by crossover or carried through complex life cycles. Such relational patterns are captured by lifting the statistical framework of Fisher from the level of individual replicators to the level of sets. Both evolutionary dynamics and inference for sets are formalized as Stochastic Stoichiometric Networks (SSNs), which provide a model of population processes undergoing co-evolutionary dynamics, and a multilevel platform reaching from genes to ecosystems. New results published this year include an analysis of the way the topology of SSNs determines their dynamics and the scaling of their fluctuations, and a new method of solution for the moments of their stationary distributions (Krishnamurthy, Smith, 2017. *J Physics A* 50: 425002, 2017; Smith, Krishnamurthy 2017. *Phys Rev E* 96: 062102). Research initiated by Betul outlines a program for reconstructing ancestral states by using phylogenetics and comparative biochemistry to resurrect ancestors of extant protein families, which can then be transformed into hosts like *E. coli* for further characterization in cellular context in the lab (Kacar et al. 2017, *Phil Trans Royal Soc Series A*). This work creates synergy between experimental evolutionary studies in the Rosenzweig lab, and efforts to reconstruct ancient carbon fixation pathways at the U Arizona and ELSI.

In conclusion, whether measured as research progress, scholarly output, media coverage, new grant submissions/ funding, invited seminars, leadership or a variety of synergistic activities, the Reliving the Past CAN-7 team has had a highly successful Year 3. We expect to achieve even more in our fourth year. We eagerly look forward to strengthening existing collaborative interactions within our team, to expanding new research initiatives across the NAI, and to engaging members of the evolution community who want to use experimental approaches to evaluate the dynamic behavior of ecological and genetic factors leading to quantum leaps in biological complexity.

Project Reports

Function by Reduction: Do Extant Symbiont Enzymes Recapitulate Ancient Metabolic Generalists?

Year 3 has been a bit transition year for the McCutcheon lab. Co-I McCutcheon gave seven invited talks in the United States, the Czech Republic, Spain, England, and Scotland. The highlight however was giving the keynote address at the Montana State Science Fair in front of hundreds of middle and high school students and their families. Much of the NASA-supported work has been foundational work, in the sense that we are working towards building something new and exciting and not just "turning the crank." In particular, we continue to focus on adding cell biological and biochemical detail to our genomic work on the origin, maintenance, and extinction of symbioses. The NASA grant supported postdoctoral fellows DeAnna Bublitz and Piotr Łukasik as well as graduate students Matt Campbell and Dan Vanderpool over the last year. Each of these team members had a productive year, as evidenced by the lab's five publications in 2017. Bublitz is in many ways doing the most difficult and cutting-edge work, in particular by localizing specific proteins (including those that the result of horizontal gene transfer) the mealybug symbiosis. She is also making progress of assessing the structure of endosymbiont membranes and cell walls. In particular she is looking at how (and if) peptidoglycan is made in endosymbionts and whether degraded endosymbionts still contain membranes with the bacterial-specific lipid cardiolipin (CL). We are very excited by preliminary data showing that in fact some endosymbionts completely lack CL in their membranes, further supporting our hypothesis that endosymbiont membranes are completely synthesized by the host. In our effort to communicate our results to the public, I am pleased to say that a movie we made late last year on our lichen work was the winner of the inaugural International Wildlife Film Festival film lab, a finalist at the Jackson Hole Wildlife Film Festival, and was featured in National Geographic's Short Film Showcase. Finally, co-I McCutcheon was elected to the Editorial Board at *Current Biology*.

Our major research goals for the next year are (a) our first paper on protein localization and cell biology in mealybugs and cicadas, (b) a paper on the transcription and population dynamics of the cicada symbiosis, and (c) work out the endosymbiont distribution dynamics in cicadas.

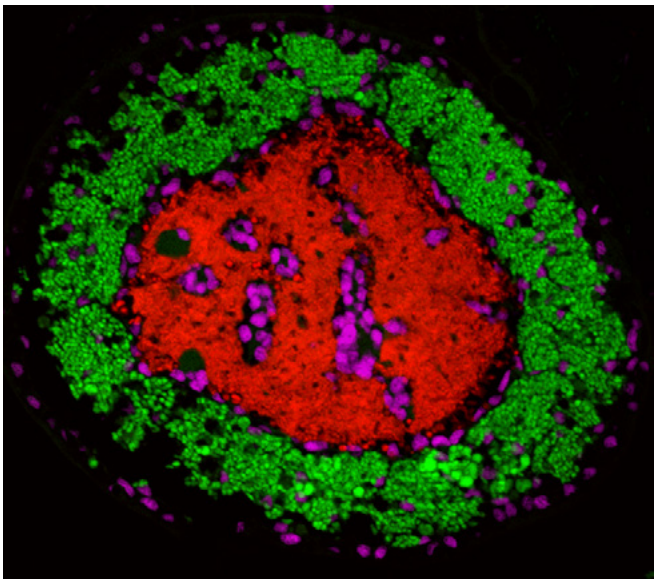


Fig. 1. A section of tissue from a cicada. Cicadas require the presence of two bacteria (red and green cells; cicada nuclei are magenta) that live in special insect cells to survive. In some cicadas, the red bacterium, called Hodgkinia, has split into two or more related species that need to function together. The genomic complexity that arises from this process mimics that seen in many mitochondria. Credit: James Van Leuven

Fig. 2. Claudio Veloso, a professor of biology at the University of Chile and collaborator of NAI co-I McCutcheon, searches the Chilean Andes for cicadas. Credit: John McCutcheon

Adaptation, Mutation Supply, and Evolution of Synergy in Biofilm Communities

Our project studies how bacterial growth in biofilms can generate diversity and multicellular behavior, and specifically how these dynamics depend upon available resources, competition within and among species, and the mutation rate of the evolving population. Our 2017 accomplishments are as follows. First, Caroline Turner, a NASA Postdoctoral Fellow, analyzed the population-genomic sequences from her experiment in which she evolved Burkholderia cenocepacia bacteria under a factorial design with high and low carbon availability and selection for biofilm and planktonic growth. She found that populations that evolved in treatments with a shared environmental factor are more genetically similar than populations which evolved in more dissimilar treatments. However, under low carbon conditions, there was almost no genetic similarity between populations evolved under biofilm and planktonic conditions, perhaps due to stronger tradeoffs between selection for biofilm and planktonic growth when carbon is scarce. In 2018, Dr. Turner will study how resource limitation affects ecological diversity in these populations (see diversity in colony morphology in Fig. 3.

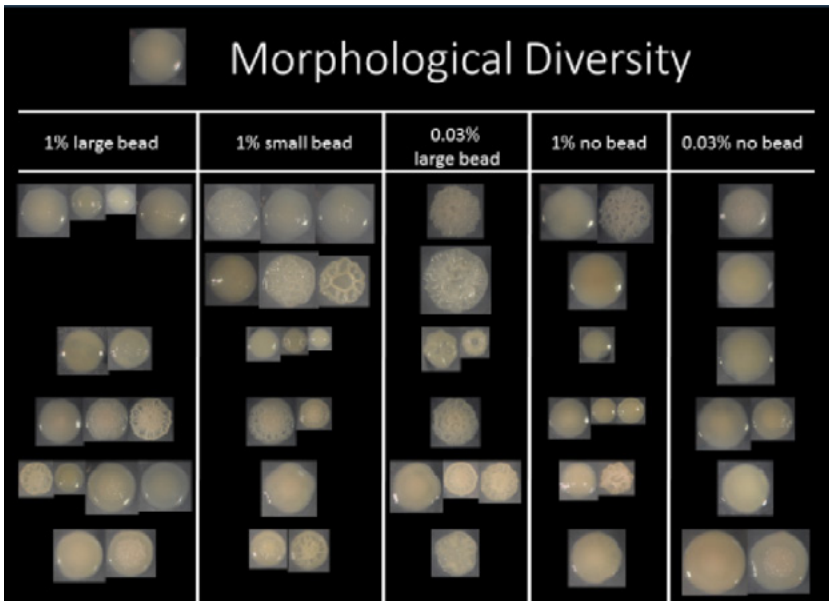


Fig. 3. The evolution of ecological diversity, represented by colony morphology, within replicate populations evolved under conditions that vary in nutrient abundance (0.03% or 1% galactose) or space for attachment (no bead, small bead, large bead).

Second, we studied the evolution of interactions between species commonly found in biofilms that compete for space and resources, *Pseudomonas aeruginosa* and *B. cenocepacia*. Recently, Dr. Chris Marshall demonstrated that, consistent with the literature, *P. aeruginosa* outcompetes *B. cenocepacia* initially, but they quickly coevolve to invade the other when rare, which suggests ecological coexistence. We are currently investigating the ecological and molecular mechanisms that enable this coexistence.

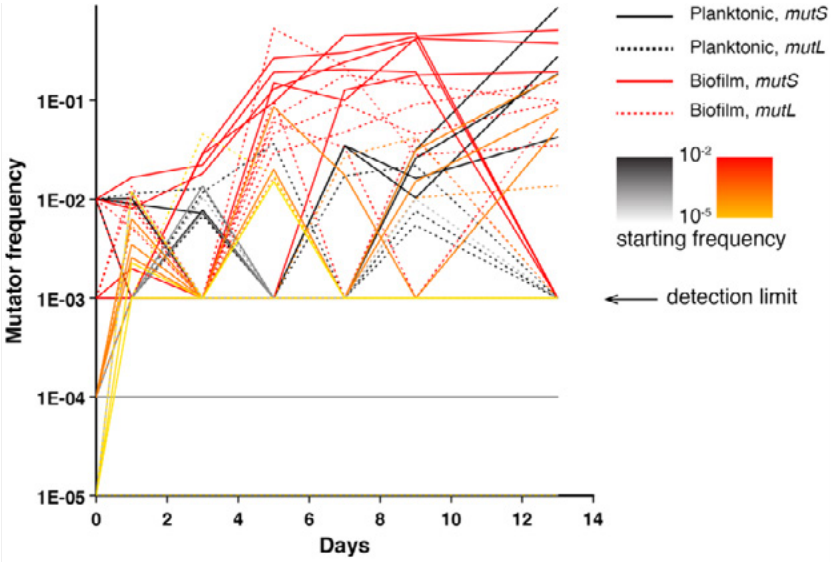


Fig. 4. Mutator genotypes with defects in mismatch repair (mutS, which increases the mutation rate ~100 fold, and mutL, which increases the rate ~50-fold) invade more predictably and from lower frequencies in biofilm populations than planktonic populations, indicating that biofilms ultimately select for lineages that acquire multiple adaptive mutations.

Third, Ph.D. student Katrina Harris tested the hypothesis that mutator genotypes may be favored and even be the source of synergistic interactions within *P. aeruginosa* biofilms. Our evidence shows that spatial structure enables multiple adaptive mutations to rise within the population and compete, which in turn favors mutators because acquiring multiple mutations enables escape from clonal interference. Further, we show that mutators invade populations grown in biofilm conditions from 1-2 logs lower starting frequency (10^{-4}) than in planktonic (10^{-2}) conditions (see Fig. 4), demonstrating that biofilms alter the population-genetic environment in favor of mutator lineages. In 2018 we are defining the boundary conditions that favor mutators and identifying the traits than enable these lineages to succeed.

The Evolution of Complexity via Multicellularity and Cell Differentiation

Experimental microbial evolution provides an opportunity for prospective, real-time observations of evolutionary processes that could otherwise only be inferred retrospectively. Predation by aquatic filter feeders has long been hypothesized to have driven the evolution of multicellularity1, but this has been difficult to test. We have shown that predation can drive the evolution of simple multicellular structures in a primitively unicellular organism.

In response to predation by the ciliate *Paramecium tetraurelia*, two populations of the unicellular green alga *Chlamydomonas reinhardtii* evolved simple multicellular structures. We have characterized the life cycles of these evolved multicellular algae (Fig. 5), some of which attain median sizes of eight or more cells (Fig. 6). Though the details of the evolved life cycles vary among strains, they have in common a unicellular bottleneck, suggesting that the multicellular structures are units of selection.

Whole genome sequencing of isolates from the experimental populations shows that the genetic basis of the evolved multicellular phenotype differs between populations. Bulked segregant analysis shows that multicellularity in one experimental population is associated with markers on chromosomes 2 and 14, suggesting that an epistatic interaction between an ancestral allele and a novel mutation is responsible for the multicellular phenotype.

The multicellular structures that have evolved in our experiments represent the very origins of multicellular development. Although the forms of multicellularity that have evolved are simple and apparently lacking in cellular differentiation, it is likely that most multicellular groups had similarly humble origins. In continuing evolution experiments, we have a unique opportunity to observe the evolution of development from the ground up, in real time, in a species that has never had a multicellular ancestor.

In 2018, we plan to carry out forward genetic analyses to confirm the causal role of the alleles suspected to have a role in the evolution of multicellularity. We will analyze whole genome sequence data from a broad sample of volvocine species to establish a robust phylogeny based on a large number of unlinked loci.

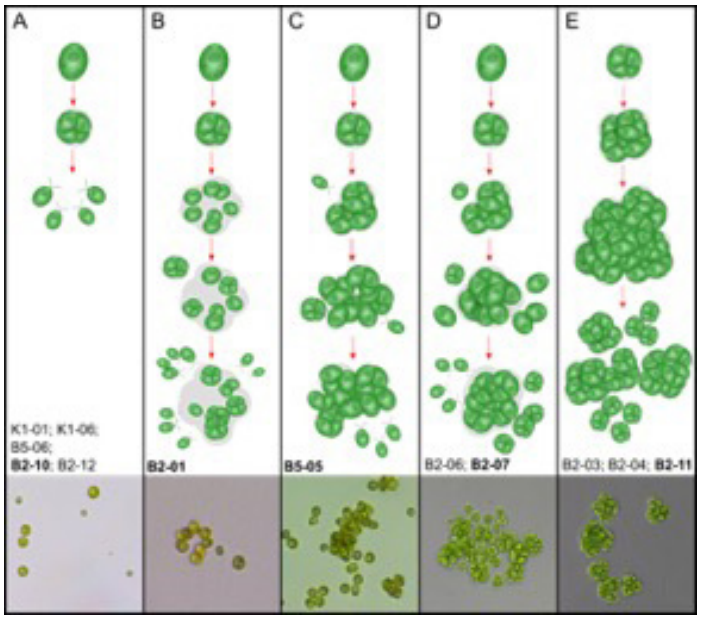


Fig. 5. Depiction of C. reinhardtii life cycles following evolution with or without predators for 350 days. Categories (A-E) show a variety of life cycle characteristics, from unicellular to various multicellular forms. Evolved strains were qualitatively categorized based on growth during 72-hour timelapse videos. Strains within each life cycle category are listed below the drawings. Representative images of each category are at the bottom (Depicted strain in bold). Strains from the B2 and B5 populations evolved with predators. Strains from the K1 population evolved without predators and approximate the wild-type life cycle.

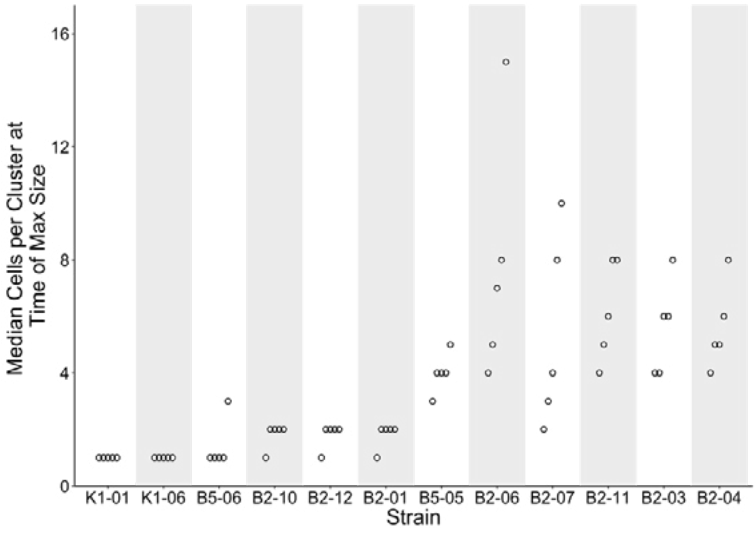


Fig. 6. Median cluster sizes of evolved strains at the time-point where strains reached maximum size. To determine the time where strains were largest, we calculated the mean from five replicate medians (open dots), for each strain and time-point. Because data are left-skewed/not normally distributed, medians were chosen to approximate central tendency. Cells per cluster were measured by sampling strains over six days of growth, staining nuclei with DAPI, and imaging using fluorescent microscopy. From left to right, time-points of maximum size for each strain were: 12, 12, 72, 120, 96, 108, 84, 72, 72, 96, 96, and 72 hours. From left to right, mean of median sizes (in cells per cluster) were: 1, 1, 1.4, 1.8, 1.8, 1.8, 4, 7.8, 5.4, 6.2, 5.6, and 5.6. Sizes at the initial time-point (0 hrs.) were omitted from analysis because they represent starting conditions. Shading is for ease of visualization.

Error Rate and the Origin and Evolution of Life: Exploring the Relationship Between Mutation, Recombination and Cooperation

Work in the Sniegowski lab at the University of Pennsylvania has focused on carrying forward projects begun in year 1 and on implementation of new experimental and theoretical approaches to the study of error rates and genetic systems. NAI collaborators have included Vaughn Cooper (University of Pittsburgh) and Philip Gerrish (Georgia Tech). NAI-supported thesis work of Tanya Singh (PhD 2016) explored the implications of deleterious mutations for the evolution and fate of populations and resulted in two peer-reviewed publications in the past year: an experimental paper describing novel findings on the relationship between mutation rate evolution and population persistence in small asexual populations of *Escherichia coli* with very high mutation rates (1; see Fig. 7), and a theoretical paper with Philip Gerrish analyzing the process whereby population lineages carrying beneficial mutations become progressively contaminated with deleterious mutations (2). Sniegowski is coauthor on a further collaborative experimental paper related to this idea that is currently in revision (Sprouffske et al., in review). NAI-supported thesis work of Mitra Eghbal (PhD 2017) explored the evolutionary instability of mutation rates in experimental populations of *E. coli*. In one project, Eghbal demonstrated that mutator populations under repeated lethal selection can evolve even-higher mutation rates (Eghbal et al., in revision), providing empirical support for the idea that error rates in adapting asexual populations have an intrinsic upward instability that can culminate in extinction (3). In a second large project, Eghbal documented mutation rate evolution in freely evolving experimental asexual mutator *E. coli* populations by propagating such populations for 1000 generations and making a large number of mutation rate measurements (Fig. 8). Genomic sequencing has identified candidate mutations affecting the mutation rate in these populations, and follow-up work is underway to establish the genetic basis and temporal dynamics of mutation rate evolution. NAI funding during 2017 supported the work of two graduate students (Mitra Eghbal and Ben Sprung), two undergraduates (Arlene Garcia and Ankur Makani), a high school URM student (Jude Dartey), and a technician (Breanna Guindon). Sniegowski is recruiting a postdoc to join the lab in the coming year.

Goals for year 4 include: 1) submission of work with Philip Gerrish describing a novel hypothesis for the evolutionary advantage of recombination; 2) submission of experimental work investigating the influence of recombination on mutation rate stability; and 3) quantifying deleterious mutation load accumulation with high-throughput fitness assays (Fig. 9).

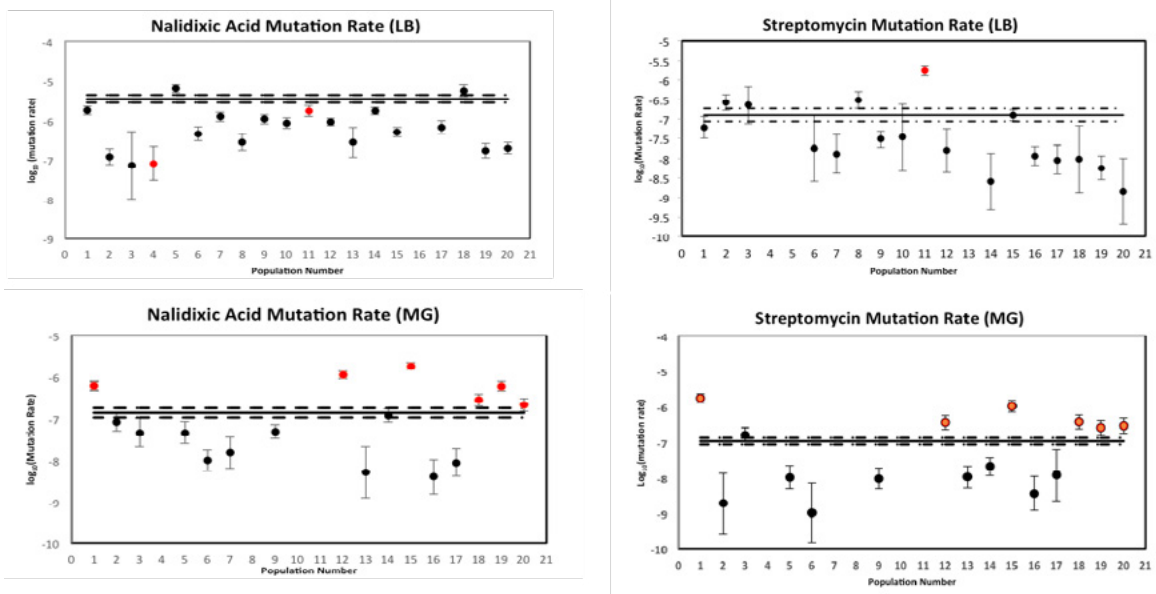


Fig. 7. Mutation rates to nalidixic acid resistance and streptomycin resistance in hypermutable experimental populations of E. coli propagated in minimal glucose (MG) or complex medium (LB) for 300 generations at very small effective size (1). Black markers denote populations that survived to the end of the experiment; red markers denote populations that went extinct. Solid horizontal line gives the estimated mutation rate in the ancestral strain; upper and lower dotted lines represent its 95% confidence interval. Evolved mutation rates were significantly higher overall in populations that went extinct than in populations that survived.

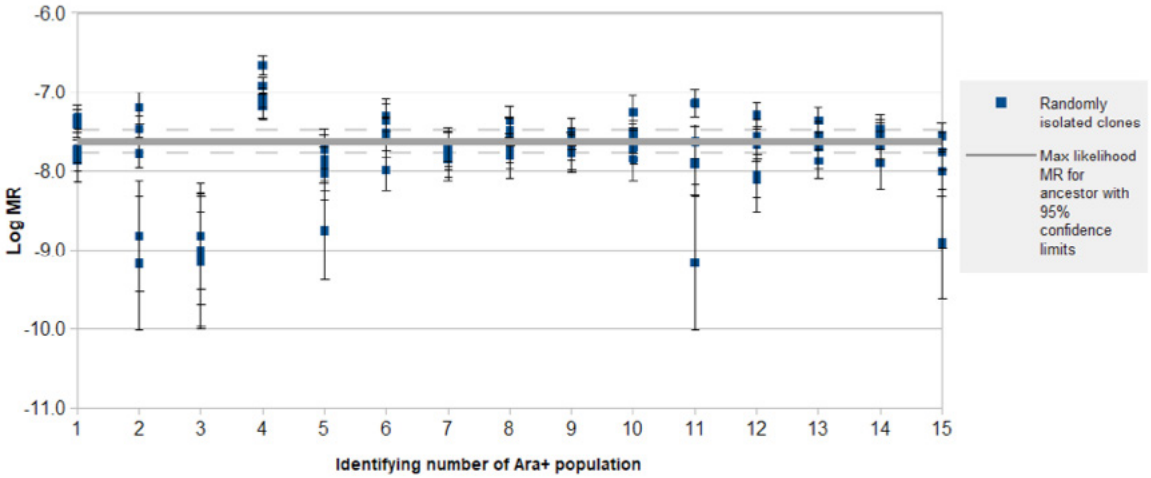


Fig. 8. Mutation rates in 15 experimental mutator populations of E. coli propagated for 1000 generations in glucose-limited medium. Horizontal solid and dashed gray lines indicate the mutation rate in the ancestral strain. Solid symbols and error bars give mutation rates (to nalidixic acid resistance) estimated in five randomly isolated clones from each population. Populations 3 and 4 appear to be fixed for spontaneously originated modifiers that reduce and increase mutation rates, respectively.

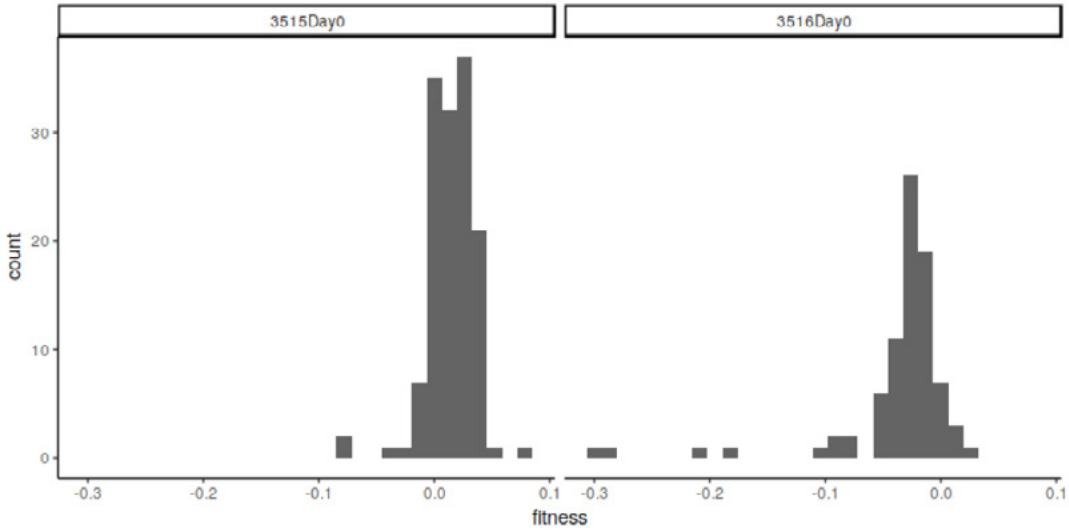


Fig. 9. Preliminary results from a novel experimental approach to analyzing the dynamics of deleterious mutation load in experimental populations of yeast (Saccharomyces cerevisiae). Populations of fluorescently marked wild-type (left histogram) and mutator (ΔMSH2; right histogram) haploid yeast were propagated for 10 generations. 138 randomly sampled clones from the wildtype population and 84 from the mutator population were then competed overnight against the ancestral strain. Frequencies of evolved and reference strains were assessed before and after competition using a benchtop FACS unit (Guava EasyCyte HT), and relative fitnesses (selection coefficients relative to the ancestor) were calculated from the observed shifts in frequency in each population. Rapid development of mutational load in the form of a leftward "tail" of reduced-fitness mutant clones (as predicted by simulations) is suggested by the data from the mutator population.

Phil Gerrish (Georgia Tech) Year 3 accomplishments include: peer-reviewed publications, including one major article in Genetics (Feb 2017); oral presentations at several national and international meetings, workshops and seminar series; induction into the Mexican Academy of Sciences (SNI-CONACyT) for PG's border-bridging work at the public university in Ciudad Juarez (UACJ); incorporation into the Georgia Tech chapter of Engineers Without Borders; national and international grant submissions; establishment of collaborative initiatives with colleagues at the NASA-affiliated Centro de Astrobiologia in Madrid, Spain. Major advances in PG's work included: a new theoretical discovery with the potential to offer an encompassing explanation for the evolution of recombination and sex; key refinements in PG's ground-breaking methodology to a) infer the distribution of fitness effects (DFE) of newly arising mutations from data taken at the population level in real time, and b) predict the near-future course of evolution. This coming year will see publication of new work entitled "On the inevitability of recombination", submission of an R01 on this topic in February, re-submission of a major ANR grant (France), and submission of a DARPA grant in collaboration with Georgia Tech faculty and Los Alamos researchers on the topic of predicting evolution.

The Origin of Robustness in Biological Major Transitions

Work by Smith in 2017 centered on lifting dynamical and statistical evolutionary concepts from the level of individuals to the level of sets and relations, to capture the hierarchical structure that arises in major transitions, and began new efforts in the reconstruction of primitive, pre-transition ancestral states.

The statistical concepts of formal evolutionary theory take three related forms: as models of stochastic dynamics; in summary-statistics such as fitness, and in the problem of inference from summary statistics to reconstruct history and identify processes [1]. Stochastic dynamics on sets is formalized within the framework of Stochastic Stoichiometric Networks (SSNs). Most familiar from Chemical Reaction Network theory [2], SSNs also provide a model of population processes undergoing coevolutionary dynamics, and a multilevel platform reaching from genes to ecosystems. New results obtained included an analysis of the way the topology of SSNs determines their dynamics and the scaling of their fluctuations, and a new method of solution for the moments of their stationary distributions [3] [4]. In another result, the Price Equation was generalized from the Fisherian concept of fitness as a property of individuals, to its arbitrary set-valued counterpart, to create a formal "population genetics of relations". Applications studied include ecological state switching, during which multi-species cohorts rather than single species determine growth or attrition of an ecosystem state, and reproduction of diploid species in which both cis- and trans-relations among genes can be targets of selection. Work is ongoing to characterize ecosystems or polyploid individuals as elementary entities using the set-valued Price Equation.

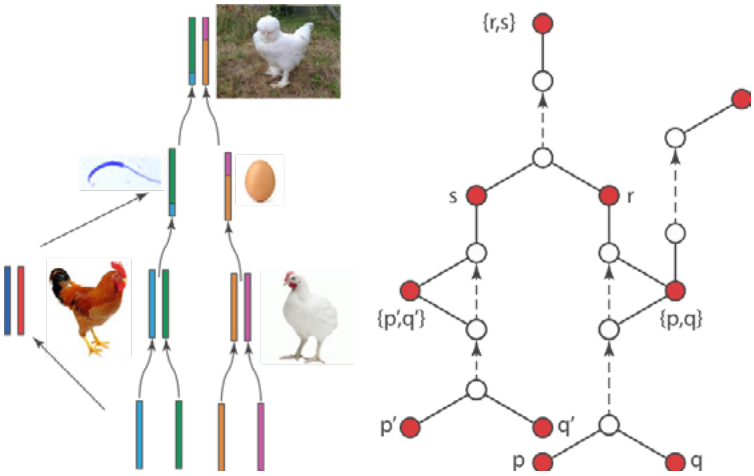


Fig. 10. Diploid heredity in the graphical notation of Stochastic Stoichiometric Networks. Biological entities receive filled dots, and events receive dashed lines. Haploid gametes combine to develop into diploid adults, which can produce new gametes with cross-over. Survival or gametogenesis may depend on other adults in the population, or adults may exit the population. Neither gametes nor adults are proper (Fisherian) replicators.

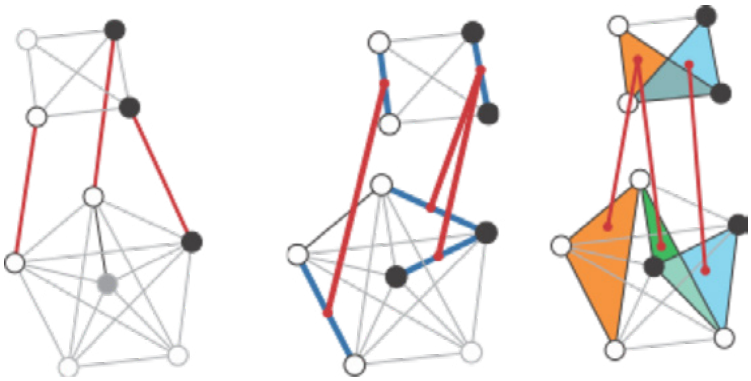


Fig. 11. Set-valued summary statistics between an ancestor generation (4 members) and a descendant generation (6 members), which take the place of individual phenotypes in the set-generalized Price Equation. Fitness is defined between ancestor individuals (dots). Summary statistics for more general correlations can be defined for pairs (links), triples (faces), or higher-ordered sets.

New work begun in 2017 outlines a program for the reconstruction of ancestral states, through a combination of phylogenetic and biochemical reconstruction of models for ancestors of extant protein families, and genome transformation of model organisms to serve as hosts for these ancestral models [5]. This work aims to take advantage of synergies between experimental microbial evolutionary studies in the Rosenzweig lab, and concurrent efforts on reconstruction of ancestral enzymes for ancient carbon fixation pathways taking place at U. Arizona and at the Earth-Life Science Institute in Japan.

Planned work in 2018 will explore the duality between dynamics and inference in SSNs, relating population change to information in evolution, and will focus heavily on the development of microbial models for ancestral state reconstruction.

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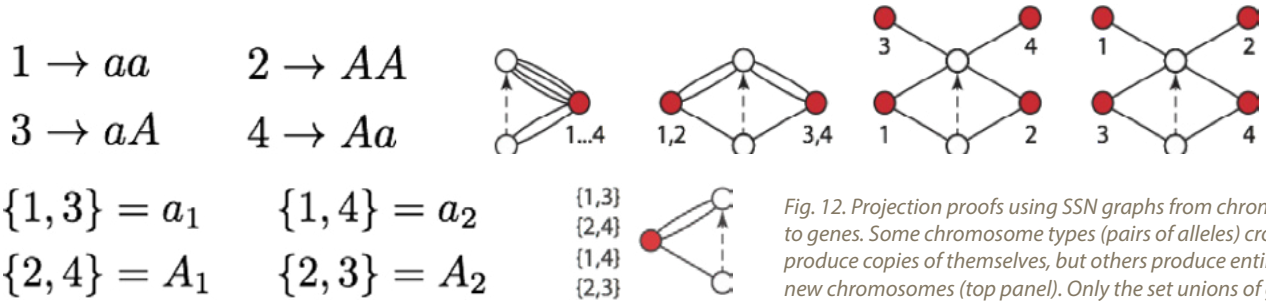

Fig. 12. Projection proofs using SSN graphs from chromosomes to genes. Some chromosome types (pairs of alleles) cross to produce copies of themselves, but others produce entirely new chromosomes (top panel). Only the set unions of graphs corresponding to single loci produce true replicators from all graphs, which is the gene level of description (bottom panel).

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Field Work

California and Oregon

Understanding both where new genes come from and the origins of organismal complexity are key goals of astrobiology. Gene duplication is an ancient mechanism that was central to both of these processes during the early evolution of life on Earth. The cyanobacterium Acaryochloris exhibits extraordinary gene duplication dynamics, and we are using this organism to develop a better understanding of the role of gene duplication for the evolution of new functions, complexity, and adaptation to novel environments. Temporal resolution of these dynamics is crucial but currently limited by the poor representation of this group in laboratory culture both within and between populations. To address this need, we have made several targeted field collections along the U. S. Pacific coast, and we are now growing multiple novel Acaryochloris strains collected from intertidal populations from California (Shelter Cove) and Oregon (Hug Point). Acaryochloris is also unique in its use of the far-red light absorbing Chlorophyll d as its primary pigment in oxygenic photosynthesis. Because the absorptive properties of this novel chlorophyll match the emission of red dwarf (M) stars, it is being used as a model by members of the VPL NAI team (Nancy Kiang, Bob Blankenship and other team members) for understanding the potential long wavelength limits of extrasolar oxygenic photosynthesis. Understanding the diversity of far-red photosynthesis within Acaryochloris is essential for this project, and our field work synergistically complements similar efforts to culture Acaryochloris from different field sites by the VPL team.

Endosymbiosis—the process by which one organism takes up residence in a host cell—is a key driver of organismal complexity. Both the mitochondrion and chloroplast were once free-living bacteria, and their establishment as endosymbiotic organelles played a critical role in the origin of all eukaryotic life. The importance of endosymbiosis is reflected in its inclusion in some of the goals of the NASA astrobiology program. But the ancient nature of organelles makes understanding the process of endosymbiosis difficult. Our lab uses a number of insect models to study how bacteria become endosymbionts, and how these processes are both similar and different to those experienced by the classic cellular organelles. These insects have very long term (10-200+ million year) relationships with bacterial endosymbionts that provide key nutrients to their hosts. We use the globally distributed signing cicadas as one of our models, because some of their endosymbionts show patterns of genome instability similar to some mitochondrial genomes. We have active field sites of cicadas from the southwestern and southeastern United States, as well as Chile, because these populations span the range of endosymbiont genome complexity within the insect group. This work, while ongoing, has already provided fundamental insight in the process of endosymbiont genome evolution (Van Leuven et al., 2014, *Cell* 158:1270-1280; Campbell et al., 2015, *PNAS* 112: 10192-10199; Campbell et al., 2017 *Curr Biol*, 27: 3568–3575; Lukasik et al., 2018, *PNAS* 115:E226-E235).

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Team Members

Frank Rosenzweig
Dimitra Aggeli
Kayli Anderson
Jacob Boswell
Stuart Brown
Deanna Bublitz
Mark Buckner
Matt Campbell
Kimberly Chen
Vaughn Cooper
Shelley Copley
Michael Cox
Bridget Creel
Mitchell Cutter
Jude Dartey
Clara Davison
Mitra Eghbal
Jake Flood
Benjamin Galeota-Sprung
Amy Gallagher
Arlene Garcia
Tyrel Garner
Philip Gerrish
Emmy Handl
Katrina Harris
Jeremy Heng
Matthew Herron
Meredeth Hyun
Andres Ferrino Iriarte
Betül Kacar
Divjot Kaur
Juhan Kim
Margie Kinnersley
John Carlo Kristofich
Reid Longley
Kennda Lynch
Ankur Makani
Chris Marshall
John McCutcheon
Hanon McShea
Scott Miller
Andrew Morgenthaler
Nate Phillips
Alex Plesa
William Ratcliff
Emiko Sano
Gavin Sherlock
Emily Sileo
Tanya Singh
Eric Smith
Paul Sniegowski
Toby Spribille
Caroline Turner
Jillian Walke
Stephanie Weldon