Annual Report for Period: 11/2002 - 10/2003
Submitted on: 10/20/2003
Principal Investigator: Lenski, Richard E.
Award ID: 9981397
Organization: Michigan State University
Title:
BIOCOMPLEXITY: Bacterial and Computational Experiments to Identify General Principles that Govern the Evolution of Complexity

Project Participants

Senior Personnel

Name: Lenski, Richard
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Riley, Margaret
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Adami, Christoph
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Ofria, Charles
Worked for more than 160 Hours: Yes
Contribution to Project:
Charles Ofrai began as a research faculty member on this project. During the past year, he was appointed as a tenure-track assistant professor at Michigan State University. As per our original proposal, he now functions effectively as a fourth co-PI on this project.

Name: Velicer, Gregory
Worked for more than 160 Hours: Yes
Contribution to Project:

Post-doc

Name: Cooper, Tim
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Moore, Francisco
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Wilke, Claus
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Remold, Susanna
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Borland, Christine

Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Campos, Paulo
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: De Oliveira, Viviane
Worked for more than 160 Hours: Yes
Contribution to Project:

Graduate Student

Name: Cooper, Vaughn
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Woods, Robert
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Ostrowski, Elizabeth
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Stredwick, Kristina
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Rozen, Daniel
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Misevic, Dusan
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Vanderhyde, James
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Teal, Tracy
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Gray, Nathan
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Collier, Travis

Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Wagenaar, Daniel
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Yedid, Gabriel
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Chow, Stephanie
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Dorn, Evan
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Forster, Robert
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Beierholm, Ulrik
Worked for more than 160 Hours: No
Contribution to Project:

Name: Drummond, Allan
Worked for more than 160 Hours: No
Contribution to Project:

Name: Hampton, Alan
Worked for more than 160 Hours: No
Contribution to Project:

Name: Stuart, Graeme
Worked for more than 160 Hours: No
Contribution to Project:

Name: Huang, Wei
Worked for more than 160 Hours: Yes
Contribution to Project:
Wei's primary research project is to study the evolution of complexity using information theoretic approaches.
She has recieved a research stipend for part of the year.

Name: Stredwick, Jason
Worked for more than 160 Hours: Yes
Contribution to Project:
Jason is doing research on methods by which populations survive at very high mutation rates.
He is also working on a sepearet digital evolution project he is studying the evolution of morphologies in digital organisms.
Jason receieved a research stipend for part of the year.

Name: Hang, Dehua
Worked for more than 160 Hours: No
Contribution to Project:

Name: Clune, Jeffory
Worked for more than 160 Hours: Yes
Contribution to Project:
Jeff is working on a variety of research projects, and helping to design an educational user interface to the Avida software.
He has been supported through the use of equiptment.

Name: Levine, Uri
Worked for more than 160 Hours: No
Contribution to Project:

Undergraduate Student

Name: Wang, Jialan
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Chunara, Rumi
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Schoenmeyr, Tor
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Berardi, Stephen
Worked for more than 160 Hours: No
Contribution to Project:

Name: Kemps, Anastasha
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Goings, Sherri
Worked for more than 160 Hours: Yes
Contribution to Project:
Sherri Goings is in charge of maintaining all of the research software under the Windows operating systems.
She received support in the form of equiptment

Name: Rupp, Matthew
Worked for more than 160 Hours: No
Contribution to Project:

Name: Wisne, Laurance
Worked for more than 160 Hours: Yes
Contribution to Project:
Larry is doing significant programming on the Avida source code.
He received summer support from the grant.

Name: Hagstrom, George
Worked for more than 160 Hours: No
Contribution to Project:

Technician, Programmer

Name: Hajela, Neerja
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Valletta, Carla
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Smith, Derek
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Austin, Nathan
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Winkworth, Cynthia
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Baer, Brain
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Nanlohy, Kaben
Worked for more than 160 Hours: Yes
Contribution to Project:

Other Participant

Research Experience for Undergraduates

Organizational Partners

California Institute of Technology

Yale University

Other Collaborators or Contacts

Prof. Michel Blot, University of Grenoble, France [deceased]
Dr. Dominique Schneider, University of Grenoble, France
Estelle Crozat, University of Grenoble, France
Prof. Santiago Elena, University of Valencia, Spain

Prof. Albert F. Bennett, University of California, Irvine
Prof. Eric Torng, Michigan State University (Computer Science)
Prof. Lee Kroos, Michigan State University (Biochemistry)
Dr. Terry Marsh, Michigan State University (Microbiology)
Prof. Robert T. Pennock, Mivhigan State University (Philosophy)
Christopher Ronnewinkel, SAP Corp., Germany
Dr. J. Arjan de Visser, Wageningen University, The Netherlands
Prof. Thomas Whittam, Michigan State University (Microbiology)
Prof. Paul D. Sniegowski, University of Pennsylvania
Dr. Philip J. Gerrish, Los Alamos National Laboratory
Dr. Christel Kamp, University of Kiel, Germany
Dr. Stefan Bornholdt, University of Kiel, Germany
Prof. Isabel Novella, Medical College Ohio (Microbiology)
Prof. Paul Turner, Yale University (Ecology & Evolutionary Biology)

Activities and Findings

Research and Education Activities:

Our Biocomplexity project has three research thrusts, each with several components. Overall, our main objective is to elucidate general principles that govern the evolutionary emergence of biocomplexity and modulate its functional consequences. One of our research thrusts employs digital organisms, which are self-replicating, mutating, and evolving computer programs, to test the generality of previous findings from biological systems. Another research thrust examines the dynamics of evolving populations of bacteria, with the objective of linking phenotypic and genetic changes. The third research thrust uses both digital organisms and bacteria to explore the emergence and consequences of ecosystem complexity. On the educational front, we aim to integrate our research with undergraduate teaching as well as graduate and postdoctoral training. We are also developing a version of the Avida software program that can better serve as a tool for teaching evolution in schools.

Findings: (See PDF version submitted by PI at the end of the report)

Training and Development:

Our projects include many opportunities for the training and development of undergraduate and graduate students, as well as postdoctoral researchers. Since this project has started, Charles Ofria has moved from a research assistant professor in the Center for Microbial Ecology at MSU to a tenure-track assistant professor in the Department of Computer Science at MSU, and he has become in all respects equivalent to a co-PI on this project. He already has several graduate and undergraduate students working in his group getting their degrees in computer science, and he is also co-advising with Richard Lenski several biology-oriented graduate students. Lenski and Ofria each taught parts of a special graduate course in the Center for Biological Modeling, and Chris Adami serves on the external advisory board of that Center. Ofria also taught an advanced seminar on the inner workings of the Avida software that was attended by several postdocs and faculty as well as by graduate students. He is now teaching a graduate-level course in computer science on digital evolution, which also has several participating undergraduate students as well as grad students from biology and philosophy. Lenski has lectured in that course, in an honors biology course, and in an undergraduate course on genomics all at MSU; and he co-teaches a portion of the core graduate course in evolution at MSU that discusses experimental approaches related to this project. MSU postdoc Susi Remold led a Genetics graduate seminar on the topic of epistasis, which is a central concept in both the bacterial and digital research projects. At Caltech, Christoph Adami has taught both his unique course on artificial life, which uses the Avida software and his own textbook on the subject, and developed a new course on evolution and biocomplexity. Several students in his courses have performed research projects that will be published in a special issue of the Artificial Life journal. Also at Caltech, undergraduate Jialan Wang was a co-author on a Nature paper that examined the influence of mutation rates on genome robustness as part of a research program for incoming freshman. Caltech postdoc Claus Wilke has brought his training from theoretical physics to bear on biological problems involving both microbial and digital systems, exemplifying interdisciplinary career development; he has interviews for tenure-track faculty positions in several types of departments, exemplifying the demand for such interdisciplinary training. Former Caltech undergraduate Dule Misevic joined the Lenski group as a graduate student at MSU and is now working on Avida projects there. Along with Ofria, he supervised research by a visiting undergraduate student, Anastasha Kemps, on the evolution of mutation rates in digital organisms. Former MSU undergraduate student Bob Woods stayed on at MSU as part of the experiments to link phenotypic and genetic changes in evolving bacterial populations. MSU computer science undergraduate Sherri Goings will enter the PhD program at MSU under the joint supervision of Ofria, a computer scientist, and Lenski, a biologist. MSU graduate student Elizabeth Ostrowski has been involved in experiments with both bacteria and digital organisms, and her current work with Avida builds directly on some experiments with bacteria. Misevic and Ostrowski have also both participated in special training courses at the University of Tennessee on the Mathematics of Biological Complexity, while Ostrowski also attended a summer Evolution course offered by the University of Fribourg, Switzerland. Gabriel Yedid

joined the MSU group as a doctoral student, having received his masters degree from one of the other biology labs so far to move into work on digital organisms, and where he had already co-authored a paper in Nature. MSU graduate student Vaughn Cooper defended his dissertation on the long-term E. coli populations; he received a prestigious postdoctoral fellowship from the University of Michigan, where he studied the evolution of pathogenic bacteria; and he is now weighing an offer for a university faculty position. Postdoc Greg Velicer finished his stay at MSU and has moved on to become a junior group leader at the Max-Planck Institute for Developmental Biology, where he continues his work on the evolution of social and developmental traits in myxobacteria that he began at MSU. At Yale, research technician Derek Smith returned to public-school teaching after spending time on the sequencing project; this hands-on research experience will enrich his ability to explain scientific principles and procedures to his students. Technician Carla Winkworth now leads the sequencing work at Yale.

The work on digital organisms, in particular, provides an ideal environment for students to learn both the intricacies of experimental work, and the rigors of the mathematical framework that describes the theoretical aspects. Students at Caltech and MSU have learned to switch from software development to using the software as a platform to perform experiments, and then using the resulting data to test important theories. Also, because evolution often produces results that vary widely because of divergent paths taken by replicate populations, each experiment must be accompanied by stringent statistical tests to weigh the significance of the results. Such training is usually only afforded the purely experimental scientists, not computational and theoretical ones. Finally, students that have participated in experiments, or used the Avida software in the class taught by Adami, come away with a sense of awe about the process of evolution: the simplicity of its principles, and the fact that it can be observed in real time, as it happens, in the computer. In an age when resistance to these scientific ideas remains widespread, this training may help improve understanding at all levels.

Outreach Activities:

Our outreach activities have included talks to minority high school students, interviews with the press about research findings, appearances on educational programs, and involvement with the emerging field of microbial forensics using perspectives gained from experimental studies of evolution.

Adami has spoken to the Outreach Program for Minority High School Youth sponsored by Caltech and the Center for Advanced Computing Research. There was a very lively response from these students, in which their initial incredulity that 'life' can be observed inside a computer gave way to pointed questions about definitions of life, and the possibility of life beyond earth.

Our research program on digital evolution has been featured in a column in Natural History magazine and, this month, in the inaugural issue of PLoS Biology. The study by Wilke et al., which appeared in Nature (2001), was reported in the San Francisco Chronicle, among various newspapers. The paper by Lenski et al., also in Nature (2003), has received considerable press coverage including such print outlets as Chronicle of Higher Education, Boston Globe, Daily Telegraph (London), Folha de Sao Paulo (Brazil), Sueddeutsche Zeitung (Germany), and Spektrum der Wissenschaft (Germany), and on the web at Astrobiology Magazine, National Geographic News, New Scientist, Space.com, Technology Research News, and Wissenschaft Online (Germany). The NSF issued a press release when our 2003 Nature paper was published, and two illustrations from this paper were chosen for inclusion in an NSF project on communicating science to the public. We are also pleased that NSF Director Rita Colwell chose to mention our research in some public talks. Adami has appeared in educational television programs, including the Discovery Channel's 'Cosmic Safari'. Adami and his work on digital life were also featured in the book that accompanied the PBS television series on Evolution. Lenski has been interviewed by reporters for Science, Discover magazine, the ScienceNOW web site, ASM News, and the web-site that accompanied the NPR show 'The DNA files.' The group's work on bacteria has been reported by ABCnews.com, ScienceNOW, and in two Nature News and Views. After the bioterrorism events of 2001, Lenski and Riley have both participated in several professional and US-government sponsored forums on microbial forensics. Lenski has also served as an advisor to some federal agencies. The close relatedness of the anthrax used in the attacks to laboratory isolates poses forensic challenges that are not unlike some of the challenges we face in tracking changes in the E. coli evolution experiment, which is one of the main foci of this grant.

Journal Publications

Velicer, G. J., L. Kroos, and R. E. Lenski., "Developmental cheating in the social bacterium Myxococcus xanthus.", Nature, p. 598, vol. 404, (2000). Published

Lenski, R. E., and G. J. Velicer., "Games microbes play.", Selection, p. 89, vol. 1, (2000). Published

Schneider, D., E. Duperchy, E. Coursange, R. E. Lenski, and M. Blot., "Long-term experimental evolution in Escherichia coli. IX. Characterization of insertion sequence-mediated mutations and rearrangements.", Genetics, p. 477, vol. 156, (2000). Published

Cooper, V. S., and R. E. Lenski., "The population genetics of ecological specialization in evolving E. coli populations.", Nature, p. 736, vol. 407, (2000). Published

C. Adami, C. Ofria, and T. C. Collier, "Evolution of biological complexity", Proc. Nat. Acad. Sci. USA, p. 4463, vol. 97, (2000). Published

C. Wilke, C. Ronnewinkel, and T. Martinetz, "Molecular evolution in dynamic fitness landscapes", Physics Reports, p. 395, vol. 349, (2001). Published

C. O. Wilke, J. L. Wang, C. Ofria, R. E. Lenski, and C. Adami.

, "Evolution of digital organisms at high mutation rate leads to survival of the flattest.", Nature, p. 331, vol. 412, (2001). Published

Cooper, V. S., D. Schneider, M. Blot, and R. E. Lenski., "Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of E. coli B.", Journal of Bacteriology, p. 2834, vol. 183, (2001). Published

Cooper, V. S., A. F. Bennett, and R. E. Lenski., "Evolution of thermal dependence of growth rate of Escherichia coli populations during 20,000 generations in a constant environment.", Evolution, p. 889, vol. 55, (2001). Published

Remold, S. K., and R. E. Lenski., "Contribution of individual random mutations to genotype-by-environment interactions in Escherichia coli.", Proceedings of the National Academy of Sciences, USA, p. 11388, vol. 98, (2001). Published

Elena, S. F., and R. E. Lenski., "Epistasis between new mutations and genetic background and a test of genetic canalization.", Evolution, p. 1746, vol. 55, (2001). Published

C. O. Wilke and C. Adami.

, "Interaction between directional epistasis and average mutational effects.", Proceedings Royal Society of London, B, p. 1469, vol. 268, (2001). Published

C. O. Wilke.

, "Adaptive evolution on neutral networks.", Bulletin of Mathematical Biology, p. 715, vol. 63, (2001). Published

C. O. Wilke and C. Ronnewinkel
, "Dynamic fitness landscapes: expansions for small mutation rates.", Physica A, p. 475, vol. 290, (2001). Published

C. O. Wilke
, "Selection for fitness vs. selection for robustness in RNA secondary structure folding.", Evolution, p. 2412, vol. 55, (2001). Published

C. Adami
, "Ab initio modeling of ecosystems with artificial life.", Natural Resource Modeling, p. 133, vol. 15, (2002). Published

P. R. A. Campos, C. Adami, and C. O. Wilke., "Optimal adaptive performance and delocalization in NK fitness landscapes", Physica A, p. 177, vol. 304, (2002). Published

C. O. Wilke., "Maternal effects in molecular evolution", Phys. Rev. Lett., p. 078101, vol. 88, (2002). Published

C. Adami and J. Chu., "Critical and near-critical branching processes
", Physical Review E, p. 011907, vol. 66, (2002). Published

C. Ofria, C. Adami, and T. C. Collier., "Design of evolvable computer languages", IEEE Trans. Evol. Comp., p. 420, vol. 6, (2002). Published

C. O. Wilke, P. R. A. Campos, and J. F. Fontanari., "The genealogical process on a correlated fitness landscape
", J. Exp. Zool., p. 274, vol. 294, (2002). Published

C. O. Wilke and C. Adami., "The biology of digital organisms", Trends in Ecology and Evolution, p. 528, vol. 17, (2002). Published

C. Adami., "What is Complexity?", BioEssays, p. 1085, vol. 24, (2002). Published

Lenski, R. E., "Come fly, and leave the baggage behind (Perspective).", Science, p. 533, vol. 294, (2001). Published

Lenski, R. E., "Twice as natural (Concepts).", Nature, p. 255, vol. 414, (2001). Published

Lenski, R. E., and M. A. Riley., "Chemical warfare from an ecological perspective (Commentary)", Proceedings of the National Academy of Sciences, USA, p. 556, vol. 99, (2002). Published

Velicer, G. J., R. E. Lenski, and L. Kroos., "Rescue of social motility lost during evolution of Myxococcus xanthus in an asocial regime", Journal of Bacteriology, p. 2719, vol. 184, (2002). Published

Schneider, D., E. Duperchy, J. Depeyrot, E. Coursange, R. E. Lenski, and M. Blot., "Genomic comparisons among Escherichia coli strains B, K-12, and O157:H7 using IS elements as molecular markers", BMC Microbiology, p. 18, vol. 2, (2002). Published

De Visser, J. A. G. M., and R. E. Lenski, "Long-term experimental evolution in Escherichia coli. XI. Rejection of non-transitive interactions as cause of declining rate of adaptation", BMC Evolutionary Biology, p. 19, vol. 2, (2002). Published

Velicer, G. J., and K. L. Stredwick., "Experimental social evolution with Myxococcus xanthus", Antonie van Leeuwenhoek, p. 155, vol. 81, (2002). Published

Cooper, V. S., "Long-term experimental evolution in Escherichia coli. X. Quantifying the fundamental and realized niche", BMC Evolutionary Biology, p. 12, vol. 2, (2002). Published

Rozen, D. E., J. A. G. M. de Visser, and P. J. Gerrish., "Fitness effects of fixed beneficial mutations in microbial populations", Current Biology, p. 1040, vol. 12, (2002). Published

A. C. Shaver, P. G. Dombrowski, J. Y. Sweeney, T. Treis, R. M. Zappala, and Paul D. Sniegowski., "Fitness evolution and the rise of mutator alleles in experimental Escherichia coli populations
", Genetics, p. 557, vol. 162, (2002). Published

Wahl, L. M., and P. J. Gerrish, "The probability that beneficial mutations are lost in populations with periodic bottlenecks", Evolution, p. 2606, vol. 55, (2001). Published

Wahl, L. M., P. J. Gerrish, and I. Saika-Voivod, "Evalutating the impact of population bottlenecks in experimental evolution", Genetics, p. 961, vol. 162, (2002). Published

C. O. Wilke and C. Adami., "Evolution of mutational robustness", Mutation Research, p. 3, vol. 522, (2003). Published

C. Kamp, C. O. Wilke, C. Adami, S. Bornholdt., "Viral evolution under the pressure of an adaptive immune system: optimal mutation rate for viral escape
", Complexity, p. 28, vol. 8, (2002). Published

Wilke, C. O., "Probability of fixation of an advantageous mutant in a viral
quasispecies", Genetics, p. 457, vol. 163, (2003). Published

Cooper, T. F., D. E. Rozen, and R. E. Lenski., "Parallel changes in gene expression after 20,000 generations of evolution in E. coli", Proceedings of the National Academy of Sciences, USA, p. 1072, vol. 100, (2003). Published

Lenski, R. E., C. L. Winkworth, and M. A. Riley., "Rates of DNA sequence evolution in experimental populations of Escherichia coli during 20,000 generations", Journal of Molecular Evolution, p. 498, vol. 56, (2003). Published

Lenski, R. E., "Phenotypic and genomic evolution during a 20,000-generation experiment with the bacterium, Escherichia coli", Plant Breeding Reviews, p. 225, vol. 24, ( ). Accepted

de Visser JAGM, Hermisson J, Wagner GP, Ancel Meyers L, Bagheri-Chaichian H, Blanchard JL, Chao L, Cheverud JM, Elena SF, Fontana W, Gibson G, Hansen TF, Krakauer D, Lewontin RC, Ofria C, Rice SH, von Dassow G, Wagner A, and Whitlock MC, "Evolution and Detection of Genetic Robustness", Evolution
, p. , vol. , ( ). Accepted

Ofria C, Adami C, and Collier TC, "Selective Pressures on Genomes in Molecular Evolution", J. theor. Biology, p. 477, vol. 222, (2003). Published

C.O. Wilke, R.E. Lenski, and C. Adami., "Compensatory mutations cause excess of antagonistic epistasis in RNA secondary structure folding", BMC Evolutionary Biology, p. 3, vol. 3, (2003). Published

C.O. Wilke, "Does the Red Queen reign in the kingdom of digital organisms?", Lect. Notes. Artif. Int., p. 405, vol. 2801, (2003). Published

C.O. Wilke and I.S. Novella, "Phenotypic mixing and hiding may contribute to memory in viral quasispecies", BMC Microbiology, p. 11, vol. 3, (2003). Published

Elena, S. F., and R. E. Lenski., "Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation.", Nature Reviews Genetics, p. 457, vol. 4, (2003). Published

Lenski, R. E., C. Ofria, R. T. Pennock, and C. Adami., "The evolutionary origin of complex features.", Nature., p. 139, vol. 423, (2003). Published

Bloom, J. D., and C. Adami., "Apparent dependence of protein evolutionary rate on number of interactions is linked to biases in protein-protein interactions data sets.
", BMC Evolutionary Biology, p. , vol. , (2003). Accepted

Books or Other One-time Publications

Lenski, R. E., "Evolution, theory and experiments.", (2000). chapter, Published
Editor(s): J. Lederberg
Collection: Encyclopedia of Microbiology
Bibliography: Academic Press, San Diego.

Lenski, R. E., "Testing AntonovicsÆ five tenets of ecological genetics: experiments with bacteria at the interface of ecology and genetics.", (2001). chapter, Published
Editor(s): M. C. Press, N. Huntly, and S. Levin
Collection: Ecology: Achievement and Challenge.
Bibliography: Blackwell Science.

D. Wagenaar and C. Adami, "Influence of chance, history and adaptation on evolution in Digitalia", (2000). proceedings, Published
Editor(s): M. Bedau, J. McCaskill, S. Rasmussen, N. Packard
Collection: Proc. of Artificial Life VII
Bibliography: MIT Press, Cambridge, MA, p. 216

C. Adami., "Simulations of evolution", (2002). chapter, Published
Editor(s): M. Pagel.
Collection: Encyclopedia of Evolution
Bibliography: Oxford University Press, New York

Lenski, R. E., "Experimental evolution: a long-term study with E. coli", (2002). chapter, Published
Editor(s): M. Pagel
Collection: Encyclopedia of Evolution
Bibliography: Oxford University Press, New York

P.R.A. Campos, C. Adami, and C.O. Wilke, "Modeling Stochastic Clonal Interference", ( ). Book Chapter, Accepted
Editor(s): G. Ciobanu
Collection: Modeling in Molecular Biology
Bibliography: Springer, in press

Web/Internet Site

URL(s):

www.dllab.caltech.edu

Description:

We have created a central web site for the Digital Life Laboratory at Caltech, from which publications, reports, and the newest version of the Avida software can be downloaded. The site also tracks media attention to the project, and prominently displays the NSF source of funding.

Other Specific Products

Product Type: Software (or netware)

Product Description:

The Avida software provides a platform for studying artificial life in the form of digital organisms, which are computer programs that can self-replicate, mutate, compete, and evolve. This software, which existed prior to this grant, is constantly being improved and extended in the course of our research. Key changes include: (a) a new tool for precisely tracing an evolutionary line of descent (completed); (b) a new tool for representing computational metabolism as genotype-phenotype arrays (completed); (c) the introduction of multiple resources useful for studying the emergence and stability of ecological communities (completed); (d) the introduction of genetic recombination (in development); and (e) the development of a more user-friendly graphical-user-interface (in progress).

Sharing Information:

Every new version of Avida becomes available, free of charge, from our website at the time that we publish a paper that uses any new enhancement.

Contributions

Contributions within Discipline:

The two principal disciplines of this project are biology and computer science. In biology, our most general contribution has been the on-going demonstration that evolution can be studied in action by employing rigorously controlled and replicated experiments. Moreover, by comparing two systems -- bacterial and digital -- we seek to better understand which features of the evolutionary emergence of biocomplexity are more general and which may be specific to a particular system. In the context of computer science, there has been growing interest in recent years in using genetic and evolutionary processes to produce complex programs. Much of this work has been done in an ad hoc manner, without designed experiments to tease apart alternative explanations and sometimes with loose analogies to biological processes. In our collaborative research we insist on careful design and analysis, and we systematically and deliberately explore biologically motivated hypotheses in a computational framework. Information on our specific contributions, including theoretical developments as well as experiments with bacteria and digital organisms, is reported in the section on research findings.

Contributions to Other Disciplines:

The theory of molecular evolution in dynamic fitness landscapes emerges from the tradition of theoretical physics. While its primary application is in evolutionary biology, the theory also has applications in condensed matter, in particular magnetic systems in temporarily varying magnetic fields.

The philosophy of science has long been interested in the theoretical and empirical bases of evolutionary biology, especially in light of perceived conflicts between science and religion in the educational realm. Our work on the emergence of complex organismal features has been stimulated by, and may contribute to, these philosophical discussions.

Contributions to Human Resource Development:

See the section on 'opportunities for training and development' for specific examples of how our project contributes to the development of human resources.

Contributions to Resources for Research and Education:

NSF support has been used to set up two Beowulf clusters, the most recent consisting of 48 machines each with dual processors. These clusters are located at MSU, and they are used for the experiments with digital organisms that are performed from Caltech as well as at MSU. As time

permits, however, these clusters may also be made available to other scientists at MSU.

Chris Adami has created a central web site (www.dllab.caltech.edu) for the Digital Life Lab at Caltech, from which publications, reports, and newest version of the Avida software can be downloaded. The site also tracks media attention to the project, and prominently displays the NSF source of funding.

Richard Lenski and Charles Ofria have been active in helping to start an disciplinary Center for Biological Modeling at MSU. The scope of the Center goes well beyond our Biocomplexity project, but our project nonetheless represents one component of it. Charles Ofria recently served on a search committee for a new faculty member now affiliated with that Center. Chris Adami was chosen as a member of the Center's external advisory board and has met with it, as well as given seminars, on multiple occasions.

Contributions Beyond Science and Engineering:

There is considerable interest in using evolutionary approaches to solve complex problems in both the computer and biological sciences. Charles Ofria and Richard Lenski met with a senior scientist at a leading software corporation, who was interested in hearing about the directions of our research with Avida. Lenski gave a keynote address at a recent conference on genetic and evolutionary computation. Lenski also visited scientists at a biotechnology company who were interested in hearing about both the bacterial and digital research. One facet of the on-going work to refine Avida, led by Chris Adami, is the potential to develop a tool useful for teaching basic concepts of evolution. Finally, the bioterrorism involving anthrax, and fears about future events involving other pathogens, have called attention to the challenges associated with the epidemiological identification of closely related microbial strains. Our research on detecting mutations and quantifying real-time rates of change in experimentally evolving populations of E. coli has relevance as a model system in this regard.

Special Requirements

Special reporting requirements: None

Change in Objectives or Scope: None

Unobligated funds: less than 20 percent of current funds

Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported:

We summarize here some findings from our NSF-funded research that were published during the fourth year of this project. Following these publications are some other recent findings and highlights. (See previous progress reports for findings from earlier years.)

The evolutionary origin of complex features. A long-standing challenge to evolutionary theory has been whether it can explain the origin of complex organismal features. We examined this issue using digital organisms – computer programs that self-replicate, mutate, compete and evolve. Populations of digital organisms often evolved the ability to perform complex logic functions requiring the coordinated execution of many genomic instructions. Complex functions evolved by building on simpler functions that had evolved earlier, provided that these were also selectively favored. However, no particular intermediate stage was essential for evolving complex functions. The first genotypes able to perform complex functions differed from their non-performing parents by only one or two mutations, but differed from the ancestor by many mutations that were also crucial to the new functions. In some cases, mutations that were deleterious when they appeared served as stepping-stones in the evolution of complex features. These findings show how complex functions can originate by random mutation and natural selection. [This work was published in *Nature* (2003) by Lenski RE, Ofria C, Pennock RT, Adami C.]

Parallel changes in gene expression after 20,000 generations of evolution in Escherichia coli. Twelve populations of *Escherichia coli*, derived from a common ancestor, evolved in a glucose-limited medium for 20,000 generations. Here we use DNA expression arrays to examine whether gene-expression profiles in two populations evolved in parallel, which would indicate adaptation, and to gain insight into the mechanisms underlying their adaptation. We compared the expression profile of the ancestor to that of clones sampled from both populations after 20,000 generations. The expression of 59 genes had changed significantly in both populations. Remarkably, all 59 were changed in the same direction relative to the ancestor. Many of these genes were members of the cAMP-cAMP receptor protein (CRP) and guanosine tetraphosphate (ppGpp) regulons. Sequencing of several genes controlling the effectors of these regulons found a nonsynonymous mutation in *spoT* in one population. Moving this mutation into the ancestral background showed that it increased fitness and produced many of the expression changes manifest after 20,000 generations. The same mutation had no effect on fitness when introduced into the other evolved population, indicating that a mutation of similar effect was present already. Our study demonstrates the utility of expression arrays for addressing evolutionary issues including the quantitative measurement of parallel evolution in independent lineages and the identification of beneficial mutations. [This work was published in *Proc. Natl. Acad. Sci. USA* (2003) by Cooper TF, Rozen DE, Lenski RE.]

Rates of DNA sequence evolution in experimental populations of *Escherichia coli* during 20,000 generations. We examined rates of DNA sequence evolution in 12 populations of *Escherichia coli* propagated in a glucose minimal medium for 20,000 generations. Previous work saw mutations mediated by mobile elements in these populations, but the extent of other genomic changes was not investigated. Four of the populations evolved defects in DNA repair and became mutators. Some 500 bp was sequenced in each of 36 genes for 50 clones, including 2 ancestral variants, 2 clones from each population at generation 10,000, and 2 from each at

generation 20,000. Ten mutations were found in total, all point mutations including mostly synonymous substitutions and nonsynonymous polymorphisms; all 10 were found in mutator populations. We compared the observed sequence evolution to predictions based on different scenarios. The number of synonymous substitutions is lower than predicted from measured mutation rates in *E. coli*, but the number is higher than rates based on comparing *E. coli* and *Salmonella* genomes. Extrapolating to the entire genome, these data predict about 250 synonymous substitutions on average per mutator population, but only about 3 synonymous substitutions per nonmutator population, during 20,000 generations. These data illustrate the challenge of finding sequence variation among bacterial isolates that share such a recent ancestor. However, this limited variation also provides a useful baseline for research aimed at finding the beneficial substitutions in these populations. [This work was published in *Journal of Molecular Evolution* (2003) by Lenski RE, Winkworth CL, Riley MA.]

Apparent dependence of protein evolutionary rate on number of interactions is linked to biases in protein–protein interactions data sets. Several studies have suggested that proteins that interact with more partners evolve more slowly. The strength and validity of this association has been called into question. Here we investigate how biases in high-throughput protein-protein interaction studies could lead to a spurious correlation. We examined the correlation between evolutionary rate and the number of protein-protein interactions for sets of interactions determined by seven different high-throughput methods in *Saccharomyces cerevisiae*. Some methods have been shown to be biased towards counting more interactions for abundant proteins, a fact that could be important since abundant proteins are known to evolve more slowly. We show that the apparent tendency for interactive proteins to evolve more slowly varies directly with the bias towards counting more interactions for abundant proteins. Interactions studies with no bias show no correlation between evolutionary rate and the number of interactions, and the one study biased towards counting fewer interactions for abundant proteins actually suggests that interactive proteins evolve more rapidly. In all cases, controlling for protein abundance significantly decreases the observed correlation between interactions and evolutionary rate. Finally, we disprove the hypothesis that small data set size accounts for the failure of some interactions studies to show a correlation between evolutionary rate and the number of interactions. The only correlation supported by a careful analysis of the data is between evolutionary rate and protein abundance. The reported correlation between evolutionary rate and protein-protein interactions cannot be separated from the biases of some protein-protein interactions studies to count more interactions for abundant proteins. [This work was published in *BMC Evolutionary Biology* (2003) by Bloom JD, Adami C.]

Selective pressures on genomes in molecular evolution. We describe the evolution of macromolecules as an information transmission process and apply tools from Shannon information theory to it. This allows us to isolate three independent, competing selective pressures that we term compression, transmission, and neutrality selection. The first two affect genome length: the pressure to conserve resources by compressing the code, and the pressure to acquire additional information that improves the channel, increasing the rate of information transmission into each offspring. Noisy transmission channels (replication with mutations) give rise to a third pressure that acts on the actual encoding of information; it maximizes the fraction of mutations that are neutral with respect to the phenotype. This neutrality selection has

important implications for the evolution of evolvability. We demonstrate each selective pressure in experiments with digital organisms. [This work was published in *Journal of Theoretical Biology* (2003) by Ofria C, Adami C, Collier TC.]

Compensatory mutations cause excess of antagonistic epistasis in RNA secondary structure folding. The rate at which fitness declines as an organism's genome accumulates random mutations is an important variable in several evolutionary theories. At an intuitive level, it might seem natural that random mutations should tend to interact synergistically, such that the rate of mean fitness decline accelerates as the number of random mutations is increased. However, in a number of recent studies, a prevalence of antagonistic epistasis (the tendency of multiple mutations to have a mitigating rather than reinforcing effect) has been observed. We studied in silico the net amount and form of epistatic interactions in RNA secondary structure folding by measuring the fraction of neutral mutants as a function of mutational distance *d*. We found a clear prevalence of antagonistic epistasis in RNA secondary structure folding. By relating the fraction of neutral mutants at distance *d* to the average neutrality at distance *d*, we showed that this prevalence derives from the existence of many compensatory mutations at larger mutational distances. Our findings imply that the average direction of epistasis in simple fitness landscapes is directly related to the density with which fitness peaks are distributed in these landscapes. [This work was published in *BMC Evolutionary Biology* (2003) by Wilke CO, Lenski RE, Adami C.]

Phenotypic mixing and hiding may contribute to memory in viral quasispecies. In a number of recent experiments with food-and-mouth disease virus, a deleterious mutant, RED, was found to avoid extinction and remain in the population for long periods of time. Since RED characterizes the past evolutionary history of the population, this observation was called quasispecies memory. While the quasispecies theory predicts the existence of these memory genomes, there is a disagreement between the expected and observed mutant frequency values. Therefore, the origin of quasispecies memory is not fully understood. We propose and analyze a simple model of complementation between the wild type virus and a mutant that has an impaired ability of cell entry, the likely cause of fitness differences between wild type and RED mutants. The mutant will go extinct unless it is recreated from the wild type through mutations. However, under phenotypic mixing-and-hiding as a mechanism of complementation, the time to extinction in the absence of mutations increases with increasing multiplicity of infection (moi). If the RED mutant is constantly recreated by mutations, then its frequency at equilibrium under selection-mutation balance also increases with increasing moi. At high moi, a large fraction of mutant genomes are encapsidated with wild-type protein, which enables them to infect cells as efficiently as the wild type virions, and thus increases their fitness to the wild-type level. Moreover, even at low moi. the equilibrium frequency of the mutant is higher than predicted by the standard quasispecies model, because a fraction of mutant virions generated from wild-type parents will also be encapsidated by wild-type protein. Our model predicts that phenotypic hiding will strongly influence the population dynamics of viruses, particularly at high moi, and will also have important effects on the mutation-selection balance at low moi. The delay in mutant extinction and increase in mutant frequencies at equilibrium may, at least in part, explain memory in quasispecies populations. [This work was published in *BMC Microbiology* (2003) by Wilke CO, Novella IS.]

Probability of fixation of an advantageous mutant in a viral quasispecies. The probability that an advantageous mutant rises to fixation in a viral quasispecies is investigated in the framework of multitype branching processes. Whether fixation is possible depends on the overall growth rate of the quasispecies that will form if invasion is successful rather than on the individual fitness of the invading mutant. The exact fixation probability can be calculated only if the fitnesses of all potential members of the invading quasispecies are known. Quasispecies fixation has two important characteristics. First, a sequence with negative selection coefficient has a positive fixation probability as long as it has the potential to grow into a quasispecies with an overall growth rate that exceeds that of the established quasispecies. Second, the fixation probabilities of sequences with identical fitnesses can nevertheless vary over many orders of magnitudes. Two approximations for the probability of fixation are introduced. Both approximations require only partial knowledge about the potential members of the invading quasispecies. The performance of these two approximations is compared to the exact fixation probability on a network of RNA sequences with identical secondary structure. [This work was published in *Genetics* (2003) by Wilke CO.]

Evolution of mutational robustness. We review recent advances in the understanding of the mutation-selection balance of asexual replicators. For over 30 years, population geneticists thought that an expression derived by Kimura and Maruyama in 1966 fully solved this problem. However, Kimura and Maruyama's result is only correct in the absence of neutral mutations. The inclusion of neutral mutations leads to a wealth of interesting new effects, and, in particular, to a selective pressure to evolve robustness against mutations. We cover recent literature on the population dynamics of asexual replicators on networks of neutral genotypes, on the out-competition of fast replicators by slower ones with better mutational support, and on the probability of fixation at high mutation rates. We discuss empirical evidence for the evolution of mutational robustness, and speculate on its relevance for higher organisms. [This review was published in *Mutation Research* (2003) by Wilke CO, Adami C.]

Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. Microorganisms have been mutating and evolving on Earth for billions of years. Now, a field of research has developed around the idea of using microorganisms to study evolution in action. Controlled and replicated experiments are using viruses, bacteria and yeast to investigate how their genomes and phenotypic properties evolve over hundreds and even thousands of generations. Here, we examine the dynamics of evolutionary adaptation, the genetic bases of adaptation, tradeoffs and the environmental specificity of adaptation, the origin and evolutionary consequences of mutators, and the process of drift decay in very small populations. [This review was published in *Nature Reviews Genetics* (2003) by Elena SF, Lenski RE.]

Progress was also made on other recent fronts, a few of which are briefly noted below:

- We have surpassed 33,000 generations in the long-term experiment with *E. coli*. In one of the 12 lines, a quite substantial and surprising metabolic change has recently occurred, and we have begun to analyze this change as a precursor to a future research proposal.

- As was noted previously, we finished sequencing randomly chosen gene regions in bacteria sampled from the long-term experiment well ahead of schedule. The sequencing effort has now turned to several candidate loci that were identified based on phenotypic changes, and this approach has also begun to yield interesting data.
- The experimental bacterial populations that we study are becoming recognized as a valuable resource for calibrating and understanding the dynamics of genomic change over time scales of thousands of cell generations. Such information is relevant to efforts in the emerging field of microbial forensics, and PI Richard Lenski has contributed his expertise to relevant efforts by the American Academy of Microbiology and various federal agencies.
- We extended our analyses of evolved changes in genome-wide patterns of gene expression to those changes caused by specific beneficial mutations that we have identified in the long-term *E. coli* lines.
- Using the Avida software for research on evolving digital organisms, we have performed experiments in which there are multiple limiting resources in order to examine the relationship between environmental productivity and species diversity. A paper reporting that work is now in preparation.
- The Avida software continues to be developed as a research platform. The potential for sexual recombination via genetic crossover has been added, and preliminary experiments performed that compare the evolutionary dynamics of asexual and sexual populations.
- A planning meeting of PIs, postdocs, graduate and undergraduate students, and collaborators was held at Caltech to discuss research progress and plans for the Avida system, including development of this software for educational use. Collaborators have been identified who are interested in, and well qualified to, take the educational applications forward.
- Our paper on the evolution of complex features using the Avida system [*Nature* (2003)] received substantial coverage in the general print and on-line press, including the issuance of a press release by NSF. Also, two figures from this paper were chosen for inclusion in an NSF staff project on communicating science to the public. Our approach of studying evolution using digital organisms was featured in the inaugural issue of *Public Library of Science Biology*.
- The PI, Richard Lenski, and a graduate student, Elizabeth Ostrowski, attended the recent NSF Biocomplexity in the Environment Awardees Meeting. Their poster summarized four projects funded by this grant, showing how they were integrated by complementary themes and approaches.