

HHMI Annual Progress Report

Grant Number: 52005125

Award Year: 2004

Institution name: Hiram College

Report Period: 09/01/2007 - 08/31/2008

Program Director: Brad Goodner

Sharing What You Have Learned

Major Outcomes:

Outcome #1. Original research embedded within courses is one of the distinctive features of our grant. During the 2007-2008 grant period, 67 different undergraduate students had a total of 213 experiences with original research projects as part of 9 different courses at Hiram College (Animal Physiology, Bioinformatics, Developmental Biology, Genetics, Introductory Biochemistry, Intermediate Organic Chemistry, Microbiology, Neuroscience, and Molecular & Cellular Biology). Over the 4 years of the grant, within-course research was incorporated into 33 total iterations of 15 different courses (the courses listed above plus Behavioral Endocrinology, Bioinformatics, Biochemistry Research Methods, Computer Simulation, Intermediate Biochemistry, Introductory Organic Chemistry, and Special Topics: Research Experience for Freshmen) that involved 197 different students in a total of 877 research-within-course contacts. Based on analysis of student assessment of 4 of these courses, 83-93% of the students agreed or strongly agreed that within-course research enhanced their learning experience, 69-97% wrote positively about the experience helping them better understand the research process, 56-78% wrote positively about the experience helping them better understand classroom topics, 76-87% wrote positively about the experience boosting their self-confidence, and 90-95% wrote positively about the experience helping them make decisions about their career choices. Most interestingly, of the vast majority who felt the experience helped them make decisions, one-fourth to one-third of them admitted that it was good to know that research was not the career for them.

Outcome #2. Our outreach efforts to high school students continued during the 2007-2008 academic year as well as in the summer. During the academic year, over 140 students from Rootstown High School, Grand River Academy, and Jefferson Area High School generated and screened over 2000 transposon insertion mutants of *Agrobacterium tumefaciens* C58 for auxotrophy, acid and base sensitivity, salt sensitivity, motility, and biofilm phenotypes. During the summer, 48 high school students from across Ohio, Pennsylvania, and Virginia participated in functional genomics research as part of a genome project on the African maize pathogen *Acidovorax avenae* pv. *avenae*. In each of two sessions of the Hiram Genomics Academy, the students learned and utilized basic microbiology skills, bacterial genetics, transposon mutagenesis, DNA isolation and cloning techniques, and some bioinformatics software to connect mutant phenotypes to the genes disrupted in the mutant strains. The efforts of these students resulted in generation and screening of over 2000 *A. avenae* transposon insertion mutants for 10 different phenotypes, and the isolation of genomic DNA and cloning out of the transposon insertion site for 40 mutants with auxotrophic, pH-sensitive, and biofilm-defective phenotypes.

Outcome #3. On April 4, 2008, the Center for Deciphering Life's Languages, one of six centers of excellence at Hiram College and the one most intimately connected to this grant, hosted its inaugural symposium entitled "Energizing Education with Genomics: Big Science Partnerships Propel Small College Advantages". The program included 11 speakers representing Howard

Hughes Medical Institute, Monsanto Company, Ganeden Biotech, Arizona State University, University of Florida, University of Michigan, a biotech venture capital firm, and other science based companies and organizations. Over 50 high school students and their teachers attended and participated in hands-on activities centered on genetics and genomics. In a poster session, 17 high school students presented work from our academic-year collaborative research outreach program, and 44 Hiram students from Genetics and Bioinformatics courses presented research progress reports from course-based research projects.

Challenges Faced by Your Program:

We continue to work on all fronts at Hiram College (government grants, nongovernment grants, development efforts) to expand our funding of summer research stipends for our undergraduates and outreach to high school students both at their home institutions and through residential research experiences at Hiram.

We have worked hard to find and hire young science faculty members who are committed to great teaching and the involvement of undergraduates in research both within and beyond courses. Supporting these new faculty members takes a lot of mentoring, additional facilities, and increased financial support.

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Student Research and Broadening Access to Science

During year 4 of our HHMI grant, 25 undergraduates at Hiram College took part in research through independent projects mentored by a total of 8 faculty and staff associated with the grant. This research effort was separate from student involvement in research within courses. In keeping with its promise of using the HHMI grant as a stepping stone to developing a consistent internal funding mechanism, Hiram College provided summer stipend support for 5 students participating in summer research, with grants to individual faculty and donor gifts supporting 16 more summer students. Based on the independent projects described in this section and on research done within courses, 11 current and former undergraduate students, supported by HHMI funds, presented their work through 5 poster presentations at 4 scientific conferences (Society for Neuroscience Conference, Midwest Primate Interest Group Meeting, Ohio Section of the American Physical Society, American Society of Mass Spectrometry Conference) and through 3 published research articles and several manuscripts in preparation or submitted. The following paragraphs describe each research project, the students and faculty involved, and any outcomes so far.

Sylvian Fissure Asymmetry in Capuchin Monkeys (*Cebus apella*)

Student: Sherry Liu

Mentors: Kimberley Phillips & Valerie Gilbert

As part of the Fall 2007 Neuroscience course, students collected data from a MRI database to address different questions in comparative neuroanatomy. One of the students in the course, Sherry Liu, investigated asymmetry of a brain feature called the Sylvian Fissure in capuchin monkeys. She continued her research after the course which led to a manuscript accepted for publication.

Outcome: Liu, S.T., & Phillips, K.A. Sylvian fissure asymmetry in capuchin monkeys. Laterality (in press).

Response Inhibition In Capuchin Monkeys

Students: Katherine Schwan, Susan Folger, & Nicole Trainer

Mentors: Kimberley Phillips & Ryan Honomichl

Inhibition is defined as the ability to ignore or suppress thought processes or actions that are irrelevant to a goal. An inhibition task used across several species is the Object Retrieval (OR) task. The animal is presented with a clear Plexiglas box baited with a food reward. The presentation of the box is varied such that the location of the box opening varies across four locations: on top, in front, or on either side of the box. Once the box is placed within reach of the animal, they often struggle to inhibit direct reaches towards the reward, regardless of the orientation of the box opening. In the present study, we test four capuchin monkeys (*Cebus apella*) on the OR task over 8 sessions. We examined reach errors on front, top and side trials. Monkeys made more errors on the side trials (54%) than on top (16%) or front trials (4%). They also made errors more often when orientation was changed from front to side or top to side. Also, errors decreased on front and side trial types from the first four to the last four sessions

(Front – 8% to 0%; Side - 66% to sd42%). We suggest that this shows the difficulty in inhibiting a direct reach response, but that improvement on the task illustrates learning effects on inhibitory function.

Outcome: Poster presentation by Katherine E. Schwan, Susan F. Folger, and Nicole Trainer at the 2008 Midwest Primate Interest Group Conference.

Prey Capture Strategies of Capuchin Monkeys (*Cebus apella*)

Student: Kaitlyn Hellner-Burris

Mentors: Kimberley Phillips & Valerie Gilbert

Morphological sex differences in the CC:brain ratio are found in capuchins, including regions involved in motor processing (anterior midbody) and spatial-ability (splenium), with males having smaller ratios than females. The functional significance of this is unclear, but may be related to behavioral laterality in cognitive and visuospatial tasks. Behavioral data investigating sex differences in spatial abilities in the context of foraging, such as prey capture, are needed to understand the functional significance of morphological differences in the corpus callosum. The purpose of this investigation was to explore prey capture strategies of brown capuchins. Six subjects (female n=4; male n=2) were presented with opportunities to catch goldfish from a kiddie pool. We recorded the number of attempts, as well as which hand was used in each attempt, for a maximum of 5 minutes. Subjects performed 4.8 ± 4.3 attempts per trial and were successful in 19.6% of attempts. A bimanual strategy was used in 43% of attempts and a unimanual strategy was used in 57% of attempts. The bimanual strategy was more successful, with captures occurring in 26.6% of these attempts and only 14.3% occurring in unimanual attempts. These preliminary data suggest females used both strategies almost equally, whereas males preferred to use a unimanual strategy. Both sexes were more successful with a bimanual strategy, with a larger difference in success rate seen in the female capuchins.

Outcome: Poster presentation by Kaitlyn Hellner-Burris at the 2008 Midwest Primate Interest Group Conference.

Brain Structures Differ Among Tool Using And Non-Tool Using Capuchin Monkeys

Student: Courtney Sobieski

Mentors: Kimberley Phillips & Valerie Gilbert

In humans, structural brain differences are associated with gender, handedness, and learned motor skills such as musical performance. With professional keyboard players, differences have been reported in various regions of the brain, including the corpus callosum, motor cortex, and cerebellum. Here we investigate whether tool using and non-tool using adult capuchin monkeys (*Cebus apella*) exhibit neuroanatomical differences due to their high degree of manipulative propensities and extractive foraging habits. Tool using monkeys demonstrated proficiency in tool manipulation to retrieve a reward whereas non-tool using monkeys were never exposed to a tool or did not demonstrate learning of the task. High resolution 3T MRI scans were obtained for each subject and total brain volume, corpus callosum, and cerebellum were traced using an ROI approach. Tool using monkeys (n=9) had larger CC: brain ratios and anterior portions of the CC: brain ratios than non-tool using monkeys (n=9). Cerebellar volume (relative to total brain volume) was 11% larger in tool using monkeys. These results show that tool using monkeys demonstrate volumetric increases in regions of the brain that are consistent with those seen in musicians. We propose that these neuroanatomical differences represent plasticity to the motor demands of tool use, and may reflect structural changes in response to long-term motor skill training.

Outcome: Poster presentations by Courtney Sobieski at the 2008 Midwest Primate Interest Group Conference and at the 2008 Society for Neuroscience Conference.

Microscopic Analysis of Lateral Line Development in Zebrafish

Student: Allison Lightbody

Mentor: Eric Bauer

The lateral line is a key sensory system in fish, but its development during embryogenesis and larval development is still poorly understood. This project was designed to use anatomical and microscopic techniques, including extensive use of Hiram's new confocal fluorescence microscope (purchased largely with HHMI funds) to study the development of the lateral line sensory system of zebrafish. Specifically, the project focused on the innervation pattern of individual components (neuromasts) of the lateral line and how the innervation of individual neuromasts behaved during early larval growth and development, a period of rapid addition of new neurons and new neuromasts. A secondary line of research was conducted on the anatomical and physiological responses of the lateral line sensory hair cells to several common ototoxic drugs, again making extensive use of the confocal microscope.

Metabolomics Approach to Understanding Avian Tuberculosis in an Endangered Species

Students: Claire McCarthy, Zan Woo, Heather Lucas, Jennifer Stroup, & Ryan Williams

Mentor: Jody Modarelli

The population of White Winged Wood Duck in their native Asian habitat is declining rapidly, resulting in their listing as an endangered species. Hiram College, in conjunction with the Akron Zoo, is working on a captive breeding program for this species. One reason for the rapid decline of this species is avian tuberculosis (aTB) caused by *Mycobacterium avium*, a potential inhabitant of soil-water interfaces. To better understand the nature of TB in the White Winged Wood Duck and to identify TB carriers as quickly as possible, mass spectrometry was employed to identify any novel lipid metabolites in ducks housed in a water-soil environment known to contain aTB (i.e., the North Carolina Zoo) that are not found in ducks housed in a soil-free, aTB-free environment (Hiram College). Results so far have identified one unique lipid found in ducks living in the aTB environment. This work continues.

Outcome: This work was presented in a poster at the 2008 American Society of Mass Spectrometry Conference.

Host-pathogen interactions of *Acidovorax avenae* subspecies *avenae*

Student: Shruti Kommareddy

Mentor: Stuart Gordon

The primary goal of this research was to characterize the response (resistant or susceptible) of a diverse group of inbred maize genotypes to the African maize pathogen *Acidovorax avenae* subspecies *avenae*. A genetically diverse set of 38 maize inbred lines were inoculated with *A. avenae* subsp. *avenae* to identify genotypes resistant to *A. avenae*. Twenty-five of these inbred lines are the founding parents of a large mapping population. The survivorship ranged from 0% to 88%. These data will be useful to genetically characterize the resistance of maize to *A. avenae*. The surface binding properties of *A. avenae* were also explored and it was hypothesized that the ability to bind to the host is important for pathogenesis of maize by *A. avenae*. Various binding assays were performed over a period of 24-hours to determine which genotype bound polystyrene most effectively.

Outcome: This work continues.

Identification and Characterization of *Acidovorax avenae* subspecies *avenae* Mutants

Student: Evan DaSilva, Eddie Gisemba, Dante Marimpietri, & Shruti Kommareddy

Mentors: Stuart Gordon, Kathryn Reynolds, & Brad Goodner

Acidovorax avenae subspecies *avenae* is a little known pathogen of maize and other grasses in the U.S., but it can be a significant pathogen of maize in tropical regions of Africa. To better understand how this pathogen works, a transposon mutagenesis strategy was employed using a Tn5 variant. A large scale mutagenesis and screening strategy was employed, involving Hiram College undergrads and 48 high school students who participated in the 2008 Hiram Genomics Academy sessions, that resulted in the generation and screening of over 2000 *Acidovorax avenae* subsp. *avenae* mutants for 10 phenotypes including auxotrophy, acid sensitivity, and biofilm defects. Approximately 40 mutants worthy of further characterization were identified, genomic DNA from those mutants was isolated and the site of transposon insertion was cloned out, and the DNA sequence of the transposon insertion was determined. Follow up experimentation focused on the acid sensitive and biofilm defective mutants, using growth curves, surface binding assays, and virulence assays. Outcome: This work continues.

Genetic Analysis of Six Putative Glutamine Synthetases in *Agrobacterium tumefaciens* C58

Student: Jamica Johnson, Heather Lane, & Scott Simmons

Mentors: Brad Goodner, Stuart Gordon, & Kathryn Reynolds

Glutamine synthetase (GS) catalyzes the synthesis of one amino acid, glutamine, from another, glutamate. However, it is much more than that. It is the major way that new nitrogen is assimilated into organic material. The amide group of glutamine acts as a major donor of organic nitrogen in many other biochemical reactions, so the cycling of glutamate and glutamine are crucial to the proper balance of organic nitrogen as compared to organic carbon in the cell. This balance is so crucial that one form of GS, called GSI, is highly regulated not only in terms of its synthesis but also in terms of its protein activity. Many members of the Bacteria domain and some members of the Archaea domain have a GSI and an associated regulatory cascade. The soil bacterium and plant pathogen *Agrobacterium tumefaciens* C58, an organism we have worked with a lot at Hiram, has a classic GSI and all the associated regulatory machinery. Unlike *E. coli*, *A. tumefaciens* C58 and its close relatives have several more GS enzymes as well. Based on work done in other bacteria, GSII and GSIII represent other types of GS enzyme. *A. tumefaciens* C58 has 6 genes encoding GS isoforms – 5 that cluster together including GSI and GSIII, and a very divergent GSII. What are their roles? To what extent do their roles overlap (genetic redundancy)? To answer these questions, primers were designed to allow the cloning of both an internal fragment of each gene and the entirety of each gene. The internal fragments were used to construct disruptions of each gene. The whole gene amplicons were cloned into an expression vector to be used for functional complementation studies. Outcome: This work continued in the Fall 2008 Molecular & Cellular Biology course and continues on through independent research.

Using Genomic Data to Construct New Tools for Nonculture-based Sampling for Bacteria

Student: Evan DaSilva, Eddie Gisemba, Dante Marimpietri, Shruti Kommareddy, Laura Matlock, Sarah Schoenhagen, Laxman Shrestha, Mariya Keremedchieva, Jamica Johnson, Heather Lane, & Scott Simmons

Mentors: Brad Goodner, Kathryn Reynolds, & Stuart Gordon

We now know that most bacteria cannot be cultured easily in the lab, but fortunately DNA-based technologies can allow us to sample these organisms without the need for culturing. Random sampling using 16S rRNA gene PCR or random genomic sequencing can be very powerful techniques, but sometimes one might only want to know if a particular organism or group of organisms is present in a sample. The availability of close to 1000 complete genome sequences allows for the potential to design species-, genus-, or higher order clade-specific PCR primers. Comparative genomic analyses using the Phylogenetic Profiler tool within the JGI Integrated Microbial Database were used to find genes highly conserved within a chosen clade, but not found in any other clades above a designated sequence identity level. Genes fitting these criteria were then used to design PCR primers specific for biovar 2-type Agrobacterium/Rhizobium subclade, the Agrobacterium/Rhizobium genus, the family Rhizobiaceae, the species *Pseudomonas syringae*, the species *Escherichia coli*, the family Enterobacteriaceae, and the phylum/kingdom Cyanobacteria. These primer sets now need to be tested for their real-world specificity and sensitivity.
Outcome: This work continues.

Development of Genetic Tools for Studying the Probiotic Strain *Bacillus coagulans* BC30

Student: Laura Matlock, Sarah Schoenhagen, Laxman Shrestha, & Mariya Keremedchieva

Mentors: Brad Goodner, Kathryn Reynolds, & Stuart Gordon

Probiotics are bacterial strains that are purposely eaten, in foodstuffs or in capsule form, to aid in digestion and promote overall gastrointestinal health. The positive attributes of probiotic strains are due to combinations of novel carbohydrate digestive enzymes, competition for binding to intestinal epithelia, and production of antibiotics. Ganeden Biotech, a small biotech firm in the Cleveland area, produces a FDA-approved probiotic strain of *Bacillus coagulans* BC30 that can be taken in capsule form and which has been incorporated into muffins, granola bars, and other foodstuffs. While the general health promoting impact of the BC30 strain has been clearly shown, very little is known about the molecular mechanisms involved. We are collaborating with Ganeden Biotech to sequence and annotate the BC30 genome as well as to potentially carry out functional genomics on this strain. While the sequencing is being done, we set out to develop genetic tools to use with the BC30 strain. Isolation of high quality genomic DNA was accomplished and genomic libraries constructed. Experiments are continuing on transformation, conjugation, and transposon mutagenesis protocols.

Outcome: This work continues.

Solvation potentials for flexible chain molecules in solution: On the validity of a pairwise decomposition

Student: Gregory Petersen (work done during 2006-2007)

Mentor: Mark Taylor

The effects of a solvent on the conformation of a flexible n -site solute molecule can be described formally in terms of an n -body solvation potential. Given the practical difficulty in computing such multibody potentials, it is common to carry out a pairwise decomposition in which the n -body potential is approximated by a sum of two-body potentials. Here we investigate the validity of this two-site approximation for short interaction-site chain-in-solvent systems. Using exact expressions for the conformation of an isolated chain, we construct a mapping between the full chain-in-solvent system and its solvation potential representation. We present results for both hard-sphere and square-well systems with $n=5$ that show that the two-site approximation is sufficient to completely capture the effects of an explicit solvent on chain conformation for a wide range of conditions which include varying the solvent diameter in the

hard-sphere system and varying the chain-solvent coupling in the square-well system. In all cases, a *set* of two-site potentials one for each distinct site-site pair is required. We also show that these two-site solvation potentials can be used to accurately compute a multisite intramolecular correlation function.

Outcome: Published in 2007 in **The Journal of Chemical Physics** 127:184901.

Conformation of a Polymer Chain in Explicit Solvent: A Solvation Potential Approach

Student: Sayuri Ichida (work done during 2006-2007)

Mentor: Mark Taylor

In much of the theoretical work on polymers in dilute solution the effects of solvent are treated in an implicit fashion: thus one studies an isolated chain interacting via an effective site-site potential. Although a formally exact mapping is possible between the chain-in-solvent system and a corresponding isolated effective potential-chain, this mapping involves a many-site solvation potential which is not practical to compute. Thus, one generally resorts to a two-site potential approximation. In this article we test the validity of this two-site approximation for a short hard-sphere (HS) n -mer chain ($n \geq 5$) in a HS solvent. This test involves computing a set of "exact" site-site solvation potentials for an isolated chain. These potentials are shown to reproduce the conformation of a chain in explicit solvent. We make use of these exact short-chain results (combining them with the solvent potential of mean force) to construct approximate two-site solvation potentials for long chains. Monte Carlo simulations have been performed for HS chain-in-solvent systems and for the corresponding isolated effective-potential n -mer chain with $n \geq 50$. These simulations show that our solvation potentials provide a quantitatively accurate description of the conformation of a HS chain in explicit (hard sphere) solvent.

Outcome: Published in 2007 in **Journal of Polymer Science: Part B. Polymer Physics** 45:3319-3326.

Conformation And Collapse Of A Square-Well Chain In A Square-Well Solvent & Effects Of Explicit Solvent On The Average Conformation Of A Polymer Chain In Solution

Students: Passang Dorji & Shishir Adhikari

Mentor: Mark Taylor

Interaction-site chains provide coarse-grained yet realistic models for polymers. Such models have long been used to study the collapse transition observed for polymers in dilute solution. In most of these studies, the variation in solvent quality (which drives the collapse) is treated implicitly through an effective polymer-polymer interaction and thus explicit polymer-solvent coupling is ignored. This coupling is expected to be important in the collapse transition due to the dramatic change in polymer conformation and exposure to solvent. Although a formally exact treatment of polymer-solvent coupling can be constructed, the required many-body solvation potential is not practical to compute. We have recently shown that for short chain-in-solvent systems this many-body solvation potential can be made tractable via an "exact" decomposition into a \textit{set} of two-site potentials. Here we use these exact short chain results to construct approximate two-site solvation potentials for long square-well chains in solvent. These solvation potentials are used to study the role of solvent in both driving and inhibiting chain collapse in square-well systems. This work continues.

Outcome: Preliminary data presented at 2008 Spring Meeting of the Ohio Section of the American Physical Society.

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Faculty Development

During the fourth year of our grant, 10 faculty, staff, and postdoctoral fellows at Hiram College participated in HHMI-supported activities. One participating faculty member, Carol Shreiner, and two staff members, Valerie Gilbert and Kathryn Reynolds, were new to Hiram College and the grant. The short paragraphs below outline the contributions of each faculty member. At the end, some challenges and opportunities for future faculty development are described, with an emphasis on new and future faculty development.

Eric Bauer, Assistant Professor of Biology, mentored one student in academic year independent research supported by supplies purchased with grant funds. Eric incorporated research into the lab component of the Developmental Biology course, and he worked with Valerie Gilbert, Research Teaching Associate of Biology & Psychology, to incorporate research into the lab portion of the Animal Physiology course. Unfortunately, due to family reasons totally unrelated to his academic work, Eric resigned his position at Hiram College in May, 2008, and moved to North Carolina to pursue new academic opportunities.

Valerie Gilbert, Laboratory Teaching Assistant of Biology & Psychology, joined Hiram College in August 2007. Valerie worked with Kim Phillips and Eric Bauer to incorporate research into their Neuroscience and Animal Physiology courses, respectively, and worked with Kim to mentor students in grant-supported independent research.

Colleen Fried, Professor of Chemistry, worked with Carol Shreiner to incorporate research involving the microwave organic synthesis accelerator labstation, purchased with grant funds, into the lab portions of the intermediate organic chemistry course.

Carol Shreiner, Assistant Professor of Chemistry, joined the Hiram College faculty in August 2007. Her expertise is in polymer chemistry. Carol teaches in the organic chemistry sequence as well as in the new nursing chemistry sequence and upper-level electives. During 2007-2008, she worked with Colleen Fried to incorporate research involving the microwave organic synthesis accelerator lab station, purchased with grant funds, into the lab portions of the intermediate organic chemistry course.

Brad Goodner, Associate Professor of Biology, administered the grant as program director (supported by a month of summer salary). In addition, he mentored 1 undergraduate in grant-supported independent research during the academic year and another 11 undergraduates during the summer (supported by research supply funds). Kathryn Reynolds, Research Teaching Associate of Biology, and Stuart Gordon, Teaching/Research Postdoctoral Fellow, also helped mentor most of those students. Working with Cathy Wheeler, Kathryn Reynolds, and Stuart Gordon, Brad incorporated research into the Molecular & Cellular Biology, Genetics, and Microbiology courses (supported by course research supply funds). Working with Ellen Walker, Brad incorporated research into the team-taught Bioinformatics course. Working with Kathryn Reynolds and Stuart Gordon, Brad ran an outreach research collaboration with 3 area high schools during the academic year (support by outreach funds), and 2 sessions of the Hiram

Genomics Academy for high school students during the summer (both sessions supported by outreach funds). He also led the organization and running of the kickoff symposium of the Hiram College Center for Deciphering Life's Languages entitled "Energizing Education with Genomics: Big Science Partnerships Propel Small College Advantages", which featured the involvement of undergraduates and high school students in research. During the grant year, Brad worked with collaborators and student coauthors on two new manuscripts and three grant proposals, all of which had significant components of undergraduate research and outreach to high schools. Using funds from a HHMI mini-grant, Brad worked with his co-PI Sally Elgin from Washington University in St. Louis along with Tuajuanda Jordan (HHMI) and Malcolm Campbell (Davidson University) to organize and run a workshop to identify the barriers preventing widespread involvement of undergraduates in genomics and bioinformatics research, and possible ways to break down those barriers. Finally, Brad attended the 28th Annual Crown Gall Conference (oral presentation on comparative genomics research accomplishments and a poster presentation on research accomplishments involving undergraduates and high school students), the inaugural meeting of the first cohort to participate in the SEA Phage Genomics National Research Initiative (modeled in large part on a proposal Brad submitted to HHMI during the previous grant year), the 2008 American Society of Microbiology Conference on Undergraduate Education (moderated a session featuring undergraduate involvement in genomics and bioinformatics including the HHMI SEA and the JGI national efforts on genomics and bioinformatics), the 2008 ASM/BIOQuest Workshop on Evolutionary Bioinformatics (session presenter), the pilot team preparatory and assessment workshops for the JGI national effort on microbial genome annotation (now called IMG-ACT), and he gave an invited seminar at Bowling Green State University.

Stuart Gordon, Teaching/Research Postdoctoral Fellow, worked with Cathy Wheeler, Kathryn Reynolds, and Brad Goodner to teach Molecular & Cellular Biology (lab component) in Fall 2007, and with Kathryn Reynolds and Brad Goodner to teach Microbiology (lab component) in Spring 2007. He team-taught the class component of Genetics with Brad Goodner in Spring 2007 as well as worked with Brad and Kathryn Reynolds on the lab component of the course. Stuart mentored several students throughout the academic year and the summer in independent research on the African maize pathogen *Acidovorax avenae* subspecies *avenae*. Stuart's research was also used as the focus for the 2008 Hiram Genomics Academy sessions which he helped run. Finally, Stuart participated along with Brad Goodner in the pilot team preparatory and assessment workshops for the JGI national effort on microbial genome annotation (now called IMG-ACT).

Jody Modarelli, Assistant Professor of Biology and Chemistry, served as an adjunct professor during the 2006-2007 academic year and was selected in a national search to fill the vacant biochemistry faculty position in the spring of 2007. During year 4 of the grant, Jody incorporated research into the lab component of the Introductory Biochemistry course and mentored 6 students in independent research, all supported by supplies purchased with grant funds.

Kim Phillips, Associate Professor of Biology and Psychology, mentored 5 students in grant-supported independent research during the academic year and summer (supported by research supply funds) and had an article accepted for publication with 1 student coauthor supported previously by a summer research stipend. In addition, Kim worked with Valerie Gilbert, Research Teaching Associate of Biology & Psychology, to teach Neuroscience with research incorporated into the laboratory portion of the course. Kim attended the 2008 Society for Neuroscience meeting along with one student who gave a poster presentation, and the 2008 Midwest Primate Interest Group Meeting with five students who gave poster presentations.

Mark Taylor, Associate Professor of Physics, published two research articles with students as coauthors based on previous research involving a large server cluster purchased with grant funds to serve the computational needs across the natural sciences. Mark also mentored two more students in computational physics projects and based on his success with undergraduate student-driven research, he wrote and was awarded a NSF grant to continue this work.

Ellen Walker, Professor of Computer Science, team taught the Bioinformatics course with Brad Goodner, which included novel research projects.

Cathy Wheeler, Research Teaching Associate of Biology, worked with Brad Goodner, Kathryn Reynolds, and Stuart Gordon to incorporate research into the laboratory component of Molecular & Cellular Biology course. In December 2007, after 16 years of incredible service to Hiram College, Cathy resigned her position to pursue a new opportunity with expanded responsibilities at John Carroll University, her alma mater. Her many contributions to all aspects of involvement with undergraduates and high school students during this grant are hereby acknowledge and greatly appreciated.

Kathryn Reynolds, Research Teaching Associate of Biology, joined Hiram College in August 2007. Kathryn worked with worked with Cathy Wheeler, Stuart Gordon, and Brad Goodner to teach Molecular & Cellular Biology (lab component) in Fall 2007, with Stuart Gordon and Brad Goodner to teach Microbiology (lab component) and Genetics (lab component) in Spring 2007. Kathryn also helped mentor several students in independent research projects during the summer and helped run the two sessions of the 2008 Hiram Genomics Academy. Finally, Kathryn took over the role of Web master for the grant team, the Biology Department, and the Center for Deciphering Life's Languages.

Our biggest challenge/opportunity for the future is how best to maintain and hopefully enlarge the scope of our impact across the natural sciences. In terms of maintenance, we need to fill the big hole left by the resignation of Cathy Wheeler, but the current financial situation suggests it will be 1-2 years before we can hire a replacement. We also need to mentor our newest faculty colleagues. The growing importance of the integrated research-teaching model at Hiram College directly impacted the hiring of 5 new faculty members and 2 new staff members in the natural sciences during grant year 3, and 2 additional faculty members in grant year 4. Positions in animal behavior/physiology, biochemistry, conservation biology, developmental biology, invertebrate ecology, eukaryotic genetics/molecular biology, and polymer chemistry were filled with the understanding that all of these new faculty members would bring their research into the lab components of one or more of their courses, that they would involve a number of undergraduates in independent research, and that they would eventually contribute to science outreach activities. Current grant funds were used to help renovate office and lab spaces for these new faculty members who will work with students in biology, biochemistry, biomedical humanities, chemistry, environmental studies, and neuroscience, as well as make contributions to the general education curriculum of the college. Our challenge will be to sustain support of these energetic young scientists in terms of mentoring, facilities, and some internal funding that can provide a baseline upon which they can seek out additional funding. In terms of enlarging the impact of the integrated research-teaching model, we need to find new ways to encourage more of our veteran faculty members to participate and to support their efforts both in the classroom and in the lab.

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Curriculum Development

Within-course research experience is one of the key distinctive features of our grant. In order to best prepare our students for the science of today and the future, we need them to see research early, see it often, and see it in the context of teams as well as through independent routes. Those characteristics define the use of research-within-courses to spur students into further research outside of courses. In grant year 4, 10 faculty, staff, and postdocs (Eric Bauer, Colleen Fried, Valerie Gilbert, Brad Goodner, Stuart Gordon, Jody Modarelli, Kimberley Phillips, Kathryn Reynolds, Carol Shreiner, and Ellen Walker) incorporated research projects into a total of 8 courses (Animal Physiology, Bioinformatics, Developmental Biology, Genetics, Intermediate Organic Chemistry, Introductory Biochemistry, Microbiology, Molecular & Cellular Biology, and Neuroscience) at Hiram College. These courses exposed 67 different students to research for a total of 213 experiences. Key research-within-course projects are described below.

The newest course to incorporate novel research within the lab component was Developmental Biology, which underwent a complete overhaul of its lab component during the fall of 2007. Eric Bauer and 7 undergraduates studied the expression pattern of several important developmental regulatory proteins (Eph's and Ephrins) in embryonic zebrafish, using fluorescently-tagged antibodies to the various proteins to label fixed 8hr-old to 48hr-old embryos and the confocal fluorescence microscope purchased previously with HHMI funds.

Eric Bauer and Valerie Gilbert continued to redesign the lab component of Animal Physiology in the spring of 2008, making extensive use of new electrophysiology equipment (iWorx; purchased in part with grant funds) to study the neuromuscular system of frogs and crayfish. For examples, the 16 students in the course worked in teams to design and perform neurophysiology experiments on crayfish tail sensory and motor neurons using extracellular electrophysiology techniques. Following the nerve recordings the neurons were filled with fluorescent dye and imaged using the confocal fluorescence microscope.

In the Fall 2007 Neuroscience, 12 students conducted independent research into an aspect of comparative neuroanatomy using a growing MRI database, under the supervision of Kimberley Phillips and Valerie Gilbert. One of the students in the course, Sherry Liu, continued her research on the asymmetry of the Sylvian Fissure in capuchin monkeys after the course, completing measurements of additional subjects. Her research led to a manuscript that is now accepted for publication.

Hiram College (Brad Goodner, Co-PI) is involved in the collaborative *Azotobacter vinelandii* Genome Project along with colleagues at Arizona State University, Monsanto Company, Seattle Pacific University, and Virginia Tech University. This project has as its goals the sequencing of one of most commonly studied strains of the free-living nitrogen-fixing *A. vinelandii* of the gamma-Proteobacteria. Besides the obvious interest in manipulating nitrogen fixation in hopes of lowering costs of agricultural nitrogen fertilization, *A. vinelandii* has many other interesting features such as a broad range of ploidy levels that is dependent upon the environment and some unique biochemical pathways. During the Fall 2007 semester, the project was in the final

stages of its annotation phase, where undergraduates served as the first layer of human experts to interpret what the genome is telling us. Whereas previous efforts at Hiram had focused on pathway verification in *A. vinelandii*, 50 students in the 2007 Molecular & Cellular Biology course were assigned 100 putative proteins each to determine their potential cellular localization and membrane topology, if applicable. Students used a combination of Web-based bioinformatics tools to identify putative Sec-dependent secretion signals, transmembrane domains, and outer membrane localization signals. Collectively, the students were able to analyze the entire proteome.

Student involvement in sequence annotation continued in the Spring 2008 semester when 38 students in the Genetics course worked under the supervision of Brad Goodner, Stuart Gordon, and Kathryn Reynolds to verify the genes necessary for a wide array of biochemical pathways and cellular processes in *Ammonifex degensii* as part of a new nationwide undergraduate research program led by the Joint Genome Institute of the U.S. Department of Energy. *A. degensii* is a chemoautolithotroph isolated from a thermal spring on the island of Java. Student teams of 2-4 were assigned a pathway or process and had three goals that mirrored the first three sections of course material. For "How Do Genes Work", each team had to hit the literature to find the steps of their assigned pathway/process and the most likely enzymes involved. Then, each team determined if the genome had all the necessary genes, whether any genes were missing at the bioinformatics level (function due to unknown convergence), and whether there was any evidence of gene redundancy. Based on the similarity alignment with its closest homologs, the students also confirmed or reassigned the start codon for each gene. For "How Are Genes Regulated", teams determined the genomic location and orientation of all their genes of interest, and predicted possible operons. They also searched for orthologs of known regulatory genes associated with their pathway or process. For "How Do Genes Change", students used gene-based phylogenetic trees to search through their assigned genes for those that did not have strong homologs in the closest sequenced relatives of *A. degensii*. Such findings provide the initial evidence for horizontal gene transfer events and many strong cases could be made from the students' analyses and in almost all cases the genomic source appeared to be other thermophiles, both from the Bacteria and Archaea domains, which potentially share the same habitat as *A. degensii*.

Obtaining a genome sequence and annotating it well can tell one a lot about an organism, but it also raises more questions than it answers. Luckily, it also greatly increases the tool set available to answer those questions. Undergraduate involvement in functional genomics took two forms in 2007-2008. First, for the 6th year in a row, 50 students in the Molecular & Cellular Biology course, working with Goodner, Gordon, and Reynolds, used reverse genetics over approximately 8 weeks of lab time to knock out 9 of the 11 genes encoding putative penicillin-binding proteins (PBP's). PBP's are involved in peptidoglycan synthesis and turnover which is essential for cell growth and maintenance of proper cell shape. Class A PBP's construct new peptidoglycan chains and crosslink them. Class B PBP's are only involved in cross-linking peptidoglycan chains, but do so in either a elongation-growth or septation-growth mode depending on the enzyme. The low molecular weight PBP's modify peptidoglycan chains before or after crosslinking. Finally, cell wall-localized beta-lactamases, besides their impact on antibiotic resistance, can be involved in peptidoglycan turnover. Over the course of the semester, each team of Hiram students amplified a portion of their assigned gene by PCR and cloned it, and used their cloned sequence to disrupt 4 Class A PBP genes, 2 Class B PBP genes, 2 out of the 4 low-MW PBP genes, and the 1 putative beta-lactamase. Their subsequent phenotypic analyses found significant growth defects in 1 class A PBP mutant and 1 low-MW PBP mutant, and motility changes in 6 mutants (2 became hypermotile, 4 became hypomotile).

When Stuart Gordon joined us as a HHMI teaching/research postdoctoral fellow, he worked with Brad Goodner to develop a microbial genome project that he could take wherever he ended up as a faculty member. After much brainstorming, Stuart decided to pursue the *Acidovorax avenae* subsp. *avenae* genome project. This is a pathogen of grasses that causes more problems with corn grown in Africa than it does here in the U.S. There are 3 close relatives whose genomes have been finished already, which will allow for some great comparative genomics. We are currently working with the U.S. Department of Energy Joint Genome Institute to get the *A. avenae* subsp. *avenae* genome sequenced, but meanwhile Stuart has led an effort to start up functional genomics with this organism. Students in the Spring 2008 Genetics course used a forward genetics approach to connect genes to phenotypes in *A. avenae* subsp. *avenae*. Over approximately 6 weeks, 50 students used transposon mutagenesis to conduct a screen for mutants affecting amino acid synthesis, nucleotide synthesis, vitamin/cofactor synthesis, pH sensitivity, osmoregulation, and biofilm formation. They generated and screened over 2000 mutants. Genomic DNA was isolated and the site of transposon insertion cloned out for 22 potential mutants of interest and the DNA sequence of the transposon insertion site was obtained. Example findings include an auxotroph identified with a mutation in pantoate – beta alanine ligase, involved in the synthesis of the vitamin pantothenate, and a biofilm defective mutant with a hit in a gene for a Type IV pilus assembly protein which are often involved in bacteria-surface interactions.

Sixteen students in the Spring 2008 Microbiology course, working with Brad Goodner, Stuart Gordon, and Kathryn Reynolds, expanded on previous efforts to use both culture-dependent and culture-independent (PCR amplification of 16S rRNA gene and some genus-specific genes) methods to sample the microbial community on the surface of trout lily (*Erythronium americanum*). Among culturable isolates, many strains of *Bacillus*, *Burkholderia*, *Flavobacterium*, and *Pseudomonas* were obtained along with strains of *Arthrobacter*, *Curtobacterium*, *Erwinia*, *Flavobacterium*, *Halolactibacillus*, *Hydrogenophaga*, *Kurthia*, *Microbacterium*, *Micrococcus*, *Paenibacillus*, and *Staphylococcus* were found. Culture-independent methods confirmed the presence of a wide diversity of *Bacillus*, *Burkholderia*, *Flavobacterium*, and *Pseudomonas* strains, as well as the presence of additional diversity within the beta-Proteobacteria that was not revealed by culture-based sampling.

Eighteen students in the Fall 2007 Introductory Biochemistry course worked under the supervision of Jody Modarelli to follow up on a research project started the preceding summer. Jody and her students, in collaboration with Dr. Gary Riggs at the Akron Zoo and Professor Chrys Wesdemiotis at The University of Akron, used mass spectrometry to look for avian TB-associated changes in the lipid profiles of the endangered White Winged Wood Duck. The purpose of the study was to verify results obtained by summer 2007 research students, who tentatively identified a unique lipid in the plasma of ducks housed in a diseased environment at the North Carolina Zoo. The class results validated previous results that a peak at m/z 715 was elevated in most of the extracted lipid samples from birds housed in the avian TB-infested environment, but that same peak was extremely low or absent in birds housed at the Hiram College Field Station in a TB-free environment. Fragmentation analysis identified the lipid peak as a derivative of arachidonic acid, even though the birds showed no signs of inflammation.

Carol Shreiner and Colleen Fried supervised 32 students in individual research projects as part of the Intermediate Organic Chemistry course. Starting with various alkyl halides, students designed and carried out the syntheses of specific organic end products, complete with characterization. Students utilized SciFinder Scholar and other library and Internet sources to search the chemical literature to find appropriate reactions and purification techniques. The syntheses were carried out during the laboratory portion of the course and spanned 5 weeks,

after which the students presented their work during a poster session. Some of the students chose to design, synthesize and characterize analgesics and other organic compounds based on reactions they learned in class and vary from the alkyl halide route. Some of the equipment used included the microwave reactor purchased with HHMI funds, GC-MS and ^1H NMR (60 MHz).

Outside of the scope of this grant, but connected through our work being used as role models, within-course research projects are now popping up throughout the biology, chemistry, computer science, and environmental studies curricula on the Hiram College campus.

HHMI Annual Progress Report

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Precollege and other Outreach Programs

In grant year 4, Brad Goodner, Stuart Gordon, and Kathryn Reynolds continued their precollege and other outreach activities with support from grant funds and through assistance from the Hiram College administration. The short paragraphs below describe their activities during the academic year and the subsequent summer, according to activity categories.

Direct Involvement with High Schools

During the academic year, Brad, Stuart, and Kathryn continued a long-term outreach collaborations with Stephanie Lammlein and her biology classes at Rootstown High School (public, rural) and initiated collaborations with two other high schools, Grand River Academy (private, suburban, all male, focuses on underachievers) and Jefferson Area High School (public rural), based on the prior attendance of two of their teachers in our summer teacher workshop. As part of courses, over 140 students (104 at Rootstown H.S.) generated and screened over 2000 transposon insertion mutants of *Agrobacterium tumefaciens* C58 for auxotrophy, acid and base sensitivity, salt sensitivity, motility, and biofilm phenotypes. In parallel with their lab research, Ms. Lammlein led her Rootstown students through readings and discussions of the science behind genetically modified crops, which often involves *Agrobacterium* as a gene transfer agent, and the hopes and potential concerns that come with the use of those crops. The students also did some research on career choices involving molecular biology lab skills. On Wednesday, May 21st, 2008, students at Rootstown High School presented posters and learning quilts to members of their local community about their year-long research project. To encourage other high school-college research collaborations, Stephanie Lammlein published a short piece on her research collaborations with Hiram College (Focus on Microbiology Education 14:6-7).

During the summer, Brad, Stuart, and Kathryn ran two sessions of the Hiram Genomics Academy involving a total of 48 high school students from across Ohio, Pennsylvania, and Virginia participated in functional genomics research as part of a genome project on the African maize pathogen *Acidovorax avenae* pv. *avenae*. In each of two sessions of the Hiram Genomics Academy, the students learned and utilized basic microbiology skills, bacterial genetics, transposon mutagenesis, DNA isolation and cloning techniques, and some bioinformatics software to connect mutant phenotypes to the genes disrupted in the mutant strains. The efforts of these students resulted in generation and screening of over 2000 *A. avenae* transposon insertion mutants for 10 different phenotypes, and the isolation of genomic DNA and cloning out of the transposon insertion site for 40 mutants with auxotrophic, pH-sensitive, and biofilm-defective phenotypes. While these 48 students returned to high school for their senior year, 7 Hiram Genomics Academy "veterans" from the previous 2 years of our efforts chose to attend Hiram College as freshmen in the fall of 2007. All of them have continued on as science majors (mostly biology).

Outreach to the Broader Northeast Ohio Community

On April 4, 2008, the Center for Deciphering Life's Languages, one of six centers of

excellence at Hiram College and the one most intimately connected to this grant, hosted its inaugural symposium entitled “Energizing Education with Genomics: Big Science Partnerships Propel Small College Advantages”. The program included 11 speakers representing Howard Hughes Medical Institute, Monsanto Company, Ganeden Biotech, Arizona State University, University of Florida, University of Michigan, a biotech venture capital firm, and other science based companies and organizations. Over 50 high school students and their teachers attended and participated in hands-on activities centered on genetics and genomics. In a poster session, 17 high school students presented work from our academic-year collaborative research outreach program, and 44 Hiram students from Genetics and Bioinformatics courses presented research progress reports from course-based research projects.

At the request of the Bill & Melinda Gates Foundation and the Teaching Institute for Essential Science, Hiram College supported the application of the Cleveland Metropolitan School District to the State of Ohio for funding to create a new STEM discovery principles-based high school, which was awarded funding. Our commitment to the new MC² STEM High School, which opened its doors to its first freshman class in August, 2008, was to provide research collaborations for their teachers and summer programming for their students through the Hiram Genomics Academy. This new collaboration continues.

Outreach on a National Level

The final outreach activities, involving Brad Goodner and Stuart Gordon, were aimed at our colleagues working in undergraduate education. Brad participated for the third year in a row as an instructor in the ASM/BioQUEST Bioinformatics Workshop for undergraduate faculty. He also helped organize and run a session at the 2008 ASM Conference on Undergraduate Education focused on undergraduate involvement in genomics and bioinformatics. Brad and Stuart participated in the pilot group of institutions working with the U.S. Department of Energy Joint Genome Institute’s (JGI) new undergraduate genome annotation project. The pilot group, working under the supervision of the JGI Education Director Cheryl Kerfeld, began the annotation of the *Ammonifex degensii* genome and worked with JGI staff to develop a new student-friendly and education-oriented annotation platform. Brad also continued to serve as an advisor to the Science Education Alliance (SEA) of the Howard Hughes Medical Institute and he worked with SEA Director Tuajuanda Jordan, HHMI Professor Sally Elgin, and Malcolm Campbell to organize and run a workshop where experts in genome sequencing, genome annotation, and undergraduate education discussed actions needed to lower the barriers to more widespread involvement of undergraduates in genomics and bioinformatics research. This workshop has fostered some new collaborations and additional efforts to engage more institutions, faculty, and students (e.g., 2008 iPlant workshop).

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