

HHMI Annual Progress Report

Grant Number: 52005125

Award Year: 2004

Institution name: Hiram College

Report Period: 09/01/2006 - 08/31/2007

Program Director: Brad Goodner

Sharing What You Have Learned

Major Outcomes:

Outcome #1. During the 2006-2007 grant period, 107 different undergraduate students had a total of 226 experiences with original research projects as part of 10 different courses at Hiram College (Animal Physiology, Behavioral Endocrinology, Biochemical Research Methods, Computer Simulation, Genetics, Intermediate Biochemistry, Intermediate Organic Chemistry, Introductory Biochemistry, Microbiology, and Molecular & Cellular Biology). As one example, students in the Behavioral Endocrinology course analyzed the connection between changes in endocrine hormone levels and behavioral changes in volunteer human subjects, captive brown capuchin monkeys, and insects. As a second example, students in the Molecular & Cellular Biology and Genetics courses participated in 2nd-pass, systems-level sequence annotation of the *Azotobacter vinelandii* genome to reconstruct metabolic pathways, verify start codons, identify possible regulatory circuits, and find evidence of horizontal gene transfer events. As a final example, students in the Molecular & Cellular Biology and Genetics courses also conducted reverse and forward genetics experiments, respectively, to connect genes to phenotypes in the soil bacterium and plant pathogen *Agrobacterium tumefaciens*.

Outcome #2. Our outreach efforts to high school students continued during the academic year as well as in the summer. During the academic year, 80 students from The Andrews School and Rootstown High School collaborated with Hiram College students to generate and screen over 4000 transposon insertion mutants for the *Agrobacterium tumefaciens* genome project. Over 40 mutants with auxotrophic and pH-sensitive phenotypes were identified. In addition, 10 students from Cleveland Benedictine High School sampled various habitats at the Cuyahoga Valley National Park, isolated culturable bacterial strains, and identified them using both culture-dependent and culture-independent methods. During the summer, 37 high school students from across Ohio, Pennsylvania, Michigan, and Indiana participated in functional genomics research as part of the *Agrobacterium* genome project. 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 transposon insertion mutants of *Agrobacterium tumefaciens* C58 for 10 different phenotypes, and the isolation of genomic DNA and cloning out of the transposon insertion site for 15 mutants with auxotrophic, pH-sensitive, and biofilm-defective phenotypes. Several of the mutants first identified by high school students have been the focus of further studies by both high school and undergraduate students.

Outcome #3: The growing importance of the integrated research-teaching model (e.g., research-within-courses) at Hiram College directly impacted the hiring of 5 new faculty members in the natural sciences. Positions in animal behavior/physiology, biochemistry, conservation biology, invertebrate ecology, 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. Within their first few months of being at Hiram College, all of these new colleagues contributed to our proposal for the 2008-2012 round of HHMI science education grants.

Challenges Faced by Your Program:

As part of our revised 2004-2008 HHMI grant budget, we committed Hiram College to start working its way toward internal funding of summer research stipends for undergraduates, beginning in grant year 3. With no HHMI funds committed to summer stipends in this grant year, the college freed some operating budget funds and worked through its development office to bring in gifts that allowed 11 students to participate in summer research. The challenge for the future is to keep that momentum going.

Sharing Resources:

Goodner, B., & C. Wheeler, 2007. Functional Genomics: Using Reverse Genetics to Test Bioinformatics Predictions. Laboratory Curriculum Resource published in ASM MicrobeLibrary. Winner of the 2006 Editor's Choice Award for Curriculum Resources.

Walker, E.L., & O. Slotterbeck, 2006. Integrated Research Components: A Practical and Effective Alternative to Senior Projects. *Journal of Computing Sciences in Colleges* 22(1):72-83.

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Student Research and Broadening Access to Science

During the third year of our HHMI grant, 28 undergraduates at Hiram College took part in research through independent projects mentored by a total of 10 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 11 of the 15 students participating in summer research, with grants to individual faculty supporting the remainder of the summer students. HHMI funds were used to purchase research supplies and equipment for the 15 summer research students and the 13 students who conducted independent research during the academic year. Based on the independent projects described in this section and on research done within courses, 9 current and former undergraduate students, supported by HHMI funds, presented their work through 3 poster presentations at 2 scientific conferences (Society for Neuroscience Conference, Midwest Primate Interest Group Meeting) and through 4 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.

Sink or Swim? Bone Density as a Mechanism for Buoyancy Control in Early Cetaceans

Students: Noel-Marie Gray, Kim Kainec, Lucas Tomko, & Scott Wolfe (all 4 supported in 2005-2006, but not during the grant period reported on here)

Mentor: Sandy Madar

Previous analyses have shown that secondarily aquatic tetrapods, including whales, exhibit osteological adaptations to life in water as part of their complex buoyancy control systems. These structural specializations of bone span hyperostosis through osteoporosis. The past 15 years of paleontological effort has provided an unprecedented opportunity to examine the osteological transformation of whales as they make their transition to an obligate aquatic lifestyle over a 10-million-year period. It is hypothesized that whales manifest their osteological specialization in the same manner as extant semiaquatic and fully aquatic mammals. This study presents and analysis of the microstructural features of bone in early and late archaic cetaceans, and in a comparative sample of modern terrestrial, semiaquatic, and aquatic mammals. Bone histology was examined from the ribs of 10 fossilized individuals representing five early cetacean families, including Pakicetidae, Ambulocetidae, Protocetidae, Remintonocetidae, and Basilosauridae. Comparisons were then made with rib histology from nine genera of extant mammals including: *Odocoileus* (deer), *Bos* (cow), *Equus* (horse), *Canis* (dog), *Lutra* (river otter), *Enhydra* (sea otter), *Choeropsis* (pygmy hippo), *Trichechus* (sea cow), and *Delphinus* (dolphin). Results show that the transition from terrestrial, to semiaquatic, to obligate aquatic locomotion in archaeocetes involved a radical shift in bone function achieved by means of profound changes at the microstructural level. A surprising finding was that microstructural change predates gross anatomical shift in archaeocetes associated with swimming. Histological analysis shows that high bone density is an aquatic specialization that provides static buoyancy control (ballast) for animals living in shallow water, while low bone density is associated with dynamic buoyancy control for animals living in deep

water. Thus, there was a shift from the typical terrestrial form, to osteopetrosis and pachyosteosclerosis, and then to osteoporosis in the first quarter of cetacean evolutionary history.

Outcome: Published in 2007 in **Anatomical Record** 290:638–653.

Postcranial Analysis of Soft-shelled Turtles from the Early Eocene

Student: Steve Milcinovich

Mentor: Sandy Madar

Fossilized postcranial remains from several Early Eocene soft shelled turtles were used to assign these specimens to specific taxa. These skeletal characters were then used to bring these specimens into existing phylogenetic trees of the turtle group. The Eocene specimens appear to represent basal species for their families. The fossilized material has been described and is currently in the hands of a specialist that can corroborate our initial findings.

Outcome: The data and analysis done here will be added to a manuscript that is already written for submission to *Journal of Paleontology*.

Brain Asymmetry and Hand Preference in Capuchin Monkeys (*Cebus apella*)

Student: Holly Koslosky

Mentors: Kimberley Phillips & Courtney Buzzell

While studies of handedness and brain asymmetry have been heavily focused on neocortical structures, similar asymmetries have been found for human cerebellar regions. We investigated cerebellar asymmetries in a New World monkey (*Cebus apella*) that does not display population-level handedness. Structural magnetic resonance images were obtained from eleven brown capuchins (female $n = 4$; male $n = 7$). Asymmetries were determined for both overall cerebellar volume and two cerebellar subregions (anterior, posterior). Hand preference was determined for each subject using a bimanual coordinated task. Results indicate that asymmetry of the anterior cerebellum was negatively correlated with hand preference ($r(10) = -0.76$, $p < .05$). Right handed individuals had a greater leftward bias of the anterior cerebellum ($U(5,5) = 2.24$, $p < .05$). We examined cerebellar AQ values for population-level asymmetry by using a one-sample t-test. A population-level leftward asymmetry of the anterior cerebellum was found, ($t(10) = -2.82$, $p = .05$).

Outcome: Poster presentation by Holly Koslosky at the 2006 Society for Neuroscience Conference.

Corpus Callosum Morphology in Capuchin Monkeys Is Influenced by Sex and Handedness

Student: Alayna Lilak

Mentors: Kimberley Phillips & Courtney Buzzell

Sex differences have been reported in both overall corpus callosum area and its regional subdivisions in humans. Some have suggested this reflects a unique adaptation in humans, as similar sex differences in corpus callosum morphology have not been reported in any other species of primate examined to date. Furthermore, an association between various measurements of corpus callosum morphology and handedness has been found in humans and chimpanzees. In the current study, we report measurements of corpus callosum cross-sectional area from midsagittal MR images collected in vivo from 14 adult capuchin monkeys, 9 of which were also characterized for hand preference on a coordinated bimanual task. Adult females were found to have a significantly larger corpus callosum: brain volume ratio, rostral body, posterior midbody, isthmus, and splenium than adult males. Left-handed individuals had a larger

relative overall corpus callosum area than did right-handed individuals. Additionally, a significant sex and handedness interaction was found for anterior midbody, with right-handed males having a significantly smaller area than right-handed females. These results suggest that sex and handedness influences on corpus callosum morphology are not restricted to *Homo sapiens*.

Outcome: Published in 2007 in **PLoS ONE** 2(8):e792.

Understanding the Basics: An Investigatory Study of Aimed Throwing in Tufted Capuchin Monkeys (*Cebus apella*)

Students: Evan Tachovsky & Katie Schwann

Mentors: Kimberley Phillips & Courtney Buzzell

The purpose of the current investigation was to explore aimed throwing behavior in captive brown capuchin monkeys (*Cebus apella*). Capuchins provide an ideal model for the study of this behavior in that they are the only New World monkeys that have been observed to throw, both in the wild and in captivity. We examined the frequency of opportunistic throwing by age and type of object. In addition, the role of handedness in opportunistic throwing was investigated. Data were collected from 13 tufted capuchins housed in two colonies over the course of 9 months. Observed instances of throwing were recorded including the hand used to throw, the object thrown and the context in which the behavior occurred. Results indicate that food was the most frequently thrown item, both within groups and when individual item preferences were assessed. Hand preference while throwing was determined for subjects who threw more than 6 times. All four subjects that met this criterion showed a significant hand preference for throwing: two subjects displayed a right hand preference ($z > 1.96$, $p < .01$) and two subjects displayed a left hand preference ($z < -1.96$, $p < .01$). Finally, there was a borderline significant negative correlation between the number of throws observed and the subject's age at the time of the throw ($r = -.63$, $p = .069$). The current research is part of an ongoing investigation to assay the intentionality and strategic logic of capuchin throwing.

Outcome: This work continues, but a progress report was presented as a poster at the 2007 Midwest Primate Interest Group Meeting.

Discrimination of social odors in brown capuchins (*Cebus apella*)

Students: Becky Vellota & Katie Schwann

Mentors: Kimberley Phillips & Courtney Buzzell

The role of pheromones in social communication has been well documented in marmosets and tamarins (*Callitrichidae*) and anatomical and behavioral evidence suggests a similar role in closely related families within platyrrhines. While brown capuchins seem to rely less on olfactory communication than their platyrrhine counterparts, they frequently engage in urine washing. The role of this behavior remains unknown, but sex differences in urine washing rates suggest a role in social communication. The purpose of this study was to examine sex differences in investigative behavior to conspecific urine. It was hypothesized that a sex difference to preferred scents would exist and that more investigative behavior would be directed to unfamiliar scents than familiar scents. Subjects (male $n = 4$, female $n = 4$) were presented with urine stimuli in choice tests in four different conditions, where stimuli were randomized by familiarity and sex of the donor. Overall males investigated stimuli significantly more than females in all conditions, $F(1,6) = 15.43$, $p < .05$. Males performed more investigatory behavior toward unfamiliar male urine than to familiar male urine, $F(1, 6) = 6.31$, $p < .05$. Females did not differ in behavior directed to familiar and unfamiliar male urine. Subjects, male or female, did not show differences in behavior toward familiar and unfamiliar

female urine. These results suggest socially relevant olfactory information may be utilized more by capuchin males than females.

Outcome: This work continues, but a progress report was presented as a poster at the 2007 Midwest Primate Interest Group Meeting.

Design of Molecular Tests for MHC Genetic Diversity in Wild Macaques

Students: Lydia Berzonsky & Elias Khalil

Mentors: Michael Gumert

The major histocompatibility loci in mammals are involved in a variety of immunological processes and show tremendous genetic polymorphism in most populations. Michael Gumert has amassed a large behavioral data set from a long-term study population of wild long-tailed macaques (*Macaca fascicularis*) in Indonesia. He also collected fecal samples from his study animals and stored the samples in a variety of ways. The goal of this project was to develop protocols for isolating high quality DNA from fecal samples, to use the research literature to design PCR primers appropriate for several MHC loci, and to develop PCR conditions to obtain amplicons for subsequent sequencing. Our biggest experimental hurdle to date has been the isolation of high quality DNA from fecal samples. Once such sequences are obtained, they can be used for parentage analysis as well as for studying the possible genetic basis of several behaviors.

Outcome: This work left Hiram College when Michael Gumert resigned from his HHMI postdoctoral position after only one year and accepted a faculty position at Nanyang Technological University in Singapore.

Microscopic Analysis of Lateral Line Development in Zebrafish

Student: Allison Lightbody

Mentors: 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.

Outcome: This work continues.

Metabolomics Approach to Understanding Avian Tuberculosis in an Endangered Species

Students: Divya Balasubramanian & Heather Lucas

Mentors: 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 aTB in the White Winged Wood Duck, a multidisciplinary approach involving mass spectrometry was initiated to identify 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 ~75 % the birds housed in the aTB environment, but not found in the aTB-free environment.

Outcome: This work continues.

Metabolomics Analysis of Urine in Pre-menopausal Women

Student: Jennifer Razek

Mentors: Jody Modarelli

Metabolomics offers a multidisciplinary approach to understanding disease states such as cancer, in hopes of identifying early detection markers and possible treatment targets. For example, renal cell carcinomas (RCC) have been shown by other groups to contain altered lipid profiles, especially cholesterol esters. Males have a much higher rate of RCC than do pre-menopausal females. In preparation for a detailed metabolomics study of RCC, previous research by students in the Introductory Biochemistry course detected differences in lipids in the urine of healthy volunteer males and females. The purpose of this study was to identify what changes, if any, occurred in the fatty acids content of urine from pre-menopausal females in response to hormones. Results indicated that certain fatty acid constituents rapidly decrease at approximately day 14 of the menstrual cycle around the time of ovulation.

Outcome: This work continues.

Development of a Microbiological Monitoring Plan for Captive Waterfowl in an Enclosed Pool

Student: Evan DaSilva & Eddie Gisemba

Mentors: Brad Goodner, Cathy Wheeler, & Stuart Gordon

Hiram College, in conjunction with the Akron Zoo, is working on a captive breeding program for the endangered White Winged Wood Duck. These ducks are being housed within soil-free enclosures, in large part to limit their possible exposure to *Mycobacterium avium*, a potential inhabitant of soil-water interfaces that causes avian tuberculosis (aTB). As part of the captive breeding program, a regular microbiological monitoring plan was devised to track any changes in the microbiota of the swimming pools within the ducks' enclosures. A sampling protocol was worked out to monitor for total bacterial colony-forming units (CFUs), enteric CFUs (e.g., *E. coli*), *Mycobacterium* species in general, and *Mycobacterium avium* in particular. The first two tests are quantitative culture-based assays, but the slow growth of *Mycobacterium* prompted the development a PCR-based assay for *Mycobacterium* spp. and *M. avium*. For the time being, the PCR assay is a +/- assay but we still need to determine the lower limit of detection.

Outcome: This work continues.

Genetic Analysis of Gluconeogenesis in *Agrobacterium tumefaciens* C58

Student: Katie Hellner-Burris & Erik Wallin

Mentors: Brad Goodner & Cathy Wheeler

Agrobacterium tumefaciens C58 can grow on gluconeogenic substrates, but analysis of its complete genome sequence reveals that it lacks a key gene involved in gluconeogenesis. It appears to lack fructose-bisphosphatase (Fbpase), even though there are 7 different enzymes known to carry out this function. Previous work at Hiram College supports that there is some type of Fbpase activity in this organism, but it must involve a novel enzyme. We have taken two approaches to identify the gene encoding the novel enzyme. We first attempted to functionally complement a Fbpase- mutant of *E. coli* using a *A. tumefaciens* genomic library, but without success. We have also used a forward genetics approach by screening for mutants that cannot

grow on gluconeogenic substrates. With this approach, we have identified the glycerol transport system and glycerol kinase, as well as several genes whose connection to gluconeogenesis is not readily apparent. However, one of these genes is regulatory and is right next to a gene for an unknown monophosphatase.

Outcome: This work continues.

Genetic Analysis of the Type VI Secretion System in *Agrobacterium tumefaciens* C58

Student: Jen Strater & Emily Hagan

Mentors: Brad Goodner, Cathy Wheeler, & Stuart Gordon

Plant wounds are typically acidic, so *A. tumefaciens* must survive these conditions and there is evidence that it actually uses low pH as a surrogate signal to change gene expression. Microarray studies conducted by a colleague identified genes induced by low pH, including a large cluster of genes on the linear chromosome II. These genes, arranged in two divergent operons, encode orthologs of what is now known as a Type VI secretion system (T6SS) and a group of effector proteins respectively. Interestingly, T6SS have only been found in bacteria known to interact with eukaryotic cells, namely pathogens and symbionts. In order to better understand the role of the T6SS in the biology of *A. tumefaciens*, site-directed mutations were made in one gene of the T6SS operon and one gene of the effector operon. These mutants are currently under analysis for changes in response to low pH, virulence, biofilm formation, and several other phenotypes.

Outcome: This work continues in collaboration with several other labs at other institutions.

NMR Analysis of Osmolytes in Wildtype and Mutants Strains of *Agrobacterium tumefaciens* C58

Student: Katie Blake

Mentors: Brad Goodner

Previous work by others has shown that *A. tumefaciens* C58 accumulates glutamate and the unique sucrose derivative mannosucrose under osmotic stress. Previous work in our lab, using both forward and reverse genetics approaches, has identified several genes important for osmoregulation. In an attempt to connect these genes to the known osmolytes, we have grown wildtype and several osmotic-sensitive mutant strains of *A. tumefaciens* under normal and osmotic stress conditions, extracted and concentrated osmolytes, and with the help of colleagues at the University of Akron Department of Chemistry, subjected the osmolytes to H-NMR. Analysis of our data so far shows that we can detect the production of mannosucrose and glutamate under osmotic stress conditions, but so far we cannot connect any of our osmotic-sensitive mutants to a decreased level in either one of these osmolytes.

Outcome: This work continues.

Search for Freshwater Bacteria Harboring Proteorhodopsins

Student: Mariya Keremedchieva, Katie Hellner-Burris, & Erik Wallin

Mentors: Brad Goodner & Cathy Wheeler

The term photosynthesis usually means only one thing to most people – the chlorophyll-based process invented by members of the domain Bacteria and borrowed by some eukaryotes through endosymbiosis of a cyanobacterial strain long ago. However, if the term is simply taken to mean light-driven energy capture, then there is another major form of photosynthesis that appears to have originated in the domain Archaea – the light-driven proton pump bacteriorhodopsin. A few years ago, new bacteriorhodopsins were found in certain marine strains from the Proteobacteria group of the domain Bacteria and these proteins were called

proteorhodopsins. So far, all known proteorhodopsins have been found in marine bacteria, but the legitimate raised in the 2007 Hiram College Microbiology course is “shouldn’t we find proteorhodopsin-based photosynthesis in freshwater as well?” This project is trying to address that question using a PCR-based cloning approach. Primers for proteorhodopsins were gleaned from the research literature and generated de novo from multiple sequence alignments. So far, these primers have failed to amplify a clearly identifiable proteorhodopsin sequence from surface freshwater samples.

Outcome: This work continues.

Genetic Analysis of pH Sensitivity in *Agrobacterium tumefaciens* C58

Student: Garrett Tiemkiewicz, students in the 2006-2007 Genetics course, and high school students from The Andrews School, Rootstown High School, and the Hiram Genomics Academy

Mentors: Brad Goodner & Cathy Wheeler

Agrobacterium strains are common soil bacteria and many of these strains can cause crown gall disease on a variety of plant species. Given that *Agrobacterium* requires a pre-existing wound in order to cause disease and that the pH within a plant wound is approximately 5.5, it seems useful to better understand how *Agrobacterium* deals with big changes in pH. As part of the *Agrobacterium* genome project, close to 10,000 transposon insertion mutations in the sequenced genome of *A. tumefaciens* C58 have been characterized for several phenotypes including sensitivity to pH 5.5 and pH 10 in solid medium growth assays. This work involved both Hiram College undergraduates and high school students. Multiple acid-sensitive and a few base-sensitive mutants were analyzed in terms of the gene disrupted in the mutant and its growth phenotype in quantitative liquid growth curves. The transposon insertion site in each mutant was cloned out and sent off for sequencing using a transposon end primer. The mutants were also grown in rich liquid medium under 4 conditions (pH 5.5, 7.0, 8.5, 10.0) to find any anomalies in growth patterns. Interestingly, most of the mutants did not show a strong growth defect in liquid media at low or high pH, in contrast to the results obtained on solid medium. The reason for this discrepancy is unclear at this time. The only mutant that showed a clear growth defect in both liquid and solid media was a pH 10-sensitive mutant lacking a K⁺ efflux system.

Outcome: This work continues.

Genetic Analysis of Biofilm Formation in *Agrobacterium tumefaciens* C58

Student: Lindsey Wilson, students in the 2006-2007 Genetics course, and high school students from The Andrews School, Rootstown High School, and the Hiram Genomics Academy

Mentors: Brad Goodner & Cathy Wheeler

In forward functional genomics, mutations are introduced randomly into a population of cells and then a particular phenotype is screened for and the disrupted gene isolated, sequenced and identified. As part of the *Agrobacterium* genome project, Hiram College undergraduates and high school students generated and screened close to 10,000 transposon insertion mutants of *A. tumefaciens* C58 for several phenotypes including biofilm defects detected on a solid medium assay. Four biofilm mutants were identified the mutated gene in each was cloned out and sequenced. The mutated genes encode fructose-bisphosphate aldolase, the exopolysaccharide synthesis protein ExoY, adenosylmethionine-8-amino-7-oxononanoate amino transferase involved in biotin synthesis, and a conserved hypothetical protein. The first two hits make sense in light of the role of extracellular polysaccharides in biofilms, but the latter two hits are still a mystery. The four mutants were further characterized by microscopic inspection of individual colonies on plates and of pellicles from standing liquid

cultures, polystyrene binding assays, growth curves, and virulence assays. One striking difference found between all of the mutants and wildtype was the appearance of the pellicles formed at the air-liquid interface in standing liquid cultures. The wildtype cells appeared very tightly packed in a matrix while the mutants appeared more mucous-like and loosely bound together. The fructose-bisphosphate aldolase mutant also had a dramatically altered binding pattern to polystyrene in response to differing levels of phosphate in the medium. Outcome: This work continues, but a progress report was given as part of an oral presentation by Brad Goodner at the 27th Annual Crown Gall Conference.

Genetic Analysis of Potential Redundancy for Malate Dehydrogenase in *Agrobacterium tumefaciens* C58

Student: Becky Roemer

Mentors: Brad Goodner & Cathy Wheeler

We typically don't think about genetic redundancy in bacteria, rather we typically think of them as extremely streamlined by selective forces for rapid replication. The sequencing of several hundred bacterial genomes has exposed more potential for genetic redundancy than previously thought. However, it is just potential redundancy until tested in the lab. We have looked at several situations of potential genetic redundancy in the *Agrobacterium tumefaciens* C58 genome and this project concerns 4 genes that look to encode malate dehydrogenase (Mdh), an enzyme of the TCA cycle. One of these genes, Atu2639, is clearly the ancestral mdh gene because it is an ortholog of the only mdh in the related *Sinorhizobium meliloti* genome and it sits in an operon with other TCA cycle genes. The other three mdh genes in the *A. tumefaciens* C58 genome appear to have entered the genome by horizontal gene transfer (xenologs). To test the potential for redundancy of Mdh function, each of the mdh genes was disrupted by site-directed homologous recombination. Growth curves conducted with a variety of carbon sources shows that one of the xenologs is not required for wildtype growth, but it appears that all the other three genes, including the ancestral ortholog, contribute to Mdh activity.

Outcome: This work continues.

Initial Characterization of Nitrogen Metabolism in *A. tumefaciens* C58

Students: Nabil Abraham & Ian Bennett

Mentors: Brad Goodner, Cathy Wheeler, & Stuart Gordon

Nitrogen metabolism in *Agrobacterium* is quite complex. The organism has a wide array of mechanisms for using inorganic and organic forms of nitrogen for its basic anabolic needs as well as for anaerobic respiration (using nitrate as electron acceptor). A much more puzzling phenomenon is that *Agrobacterium* can grow in the absence of any added nitrogen in the growth medium. The intriguing possibility of nitrogen fixation/nitrogen scavenging (we favor the latter scenario) came to our doorstep in the form of *Agrobacterium* strain UK1. This strain was pulled out of a stream bank in the United Kingdom by Dr. Paul Bishop of NCSU as a potential free-living N-fixer. While its initial phenotype suggested it was *Azotobacter*, its 16S rRNA sequence said otherwise and we have shown that it is clearly a biovar 1 *Agrobacterium*. This strain and the sequenced *A. tumefaciens* C58 can grow in minimal medium minus nitrogen in aerobic and microaerophilic conditions. This project continues to seek a biochemical explanation for the nitrogen fixation/scavenging.

Outcome: This work continues.

Distribution of a Linear Second Chromosome in the Agrobacterium/Rhizobium Clade

Student: Alayna Harkleroad

Mentors: Brad Goodner & Cathy Wheeler

Agrobacterium strains are alpha-Proteobacteria capable of producing tumors in plants. They are classified within the family Rhizobiaceae, which also includes the nitrogen-fixing plant symbiont *Rhizobium*. The pathogenic vs. mutualistic lifestyles of these organisms are largely dependent on various plasmids harbored by individual strains. *Agrobacterium* strains are subdivided into three groups, called Biovars, based on physiological characteristics. In 2001, the genome sequence of the representative Biovar 1 strain *Agrobacterium tumefaciens* C58 was published by two independent groups. It has an unusual genome structure consisting of one circular and one linear chromosome, plus two plasmids. Representative genomes of Biovar 2 and 3 organisms have recently been completed by a consortium that includes our lab, and their genome structures are distinct from Biovar 1. The focus of my work has been on the distribution of the linear chromosome among the Rhizobiaceae. It is limited to Biovar 1 strains of *Agrobacterium*, but is also present in certain mutualistic symbionts classified as *Rhizobium*. The gene responsible for replication of the linear chromosome telomeres, *telA*, has been identified and it too is limited to Biovar 1 strains within the Agrobacterium/Rhizobium mixed clade.

Outcome: This work is now part of a larger genome sequence manuscript ready for submission.

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

Student: Gregory Petersen

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 many-body 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

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 ($n \approx 5$) 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 $n \approx 50$. 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.

HHMI Annual Progress Report

Grant Number: 52005125

Award Year: 2004

Institution name: Hiram College

Report Period: 09/01/2006 - 08/31/2007

Program Director: Brad Goodner

Faculty Development

During the third year of our grant, 15 faculty, staff, and postdoctoral fellows at Hiram College participated in HHMI-supported activities. Six of these colleagues are new to the grant team (Gordon, Gumert, Modarelli, Slotterbeck, Taylor, Walker). 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 summer independent research supported by supplies purchased with grant funds. Eric worked with Courtney Buzzell, Research Teaching Associate of Biology & Psychology, to incorporate research into the lab portion of the Animal Physiology course. Finally, Eric received one month of summer salary support from the grant to develop additional research-within-courses ideas for his upcoming Developmental Biology and Animal Physiology courses, and to get his research lab up and running. His research expertise is developmental neuroscience with a focus on the zebrafish model system. Finally, Eric took the lead in critiquing and selecting the best fluorescence confocal microscope for the college to purchase using mostly grant funds supplemented by funds from the college.

Courtney Buzzell, Research Teaching Associate of Biology & Psychology, worked with Kim Phillips and Eric Bauer to incorporate research into their Behavioral Endocrinology and Animal Physiology courses, respectively, and worked with Kim to mentor students in grant-supported independent research. During the summer of 2007, Courtney resigned her position at Hiram College for personal reasons and moved with her family to the Boston area. She was a great part of our grant team that we hated to lose and we wish her nothing but the best for the future. The vacant Research Teaching Associate position was filled at the start of the 4th year of the grant.

Colleen Fried, Professor of Chemistry, incorporated research involving the microwave organic synthesis accelerator labstation, purchased with grant funds, into the lab portions of the introductory and intermediate organic chemistry courses.

Brad Goodner, Associate Professor of Biology, administered the grant as program director (supported by a month of summer salary). In addition, he mentored 8 undergraduates in grant-supported independent research during the academic year and another 7 undergraduates during the summer (supported by research supply funds). Cathy Wheeler, Research Teaching Associate of Biology, and Stuart Gordon, Teaching/Research Postdoctoral Fellow, also helped mentor most of those students. Working with Cathy Wheeler and Stuart Gordon, Brad incorporated research into the Molecular & Cellular Biology, Genetics, and Microbiology courses (supported by course research supply funds). Working with Cathy Wheeler, Brad ran an outreach research collaboration with 3 area high schools during the academic year (support by outreach funds), a summer teachers workshop (supported by outreach funds), and 2 sessions of the Hiram Genomics Academy for high school students during the summer (both sessions

supported by outreach funds). In January of 2007, Brad was named director of the new Center for Deciphering Life's Languages at Hiram College that would focus on the involvement of undergraduates and high school students in a wide range of molecular science research experiences, and he was installed as the second holder of the Edward J. Smerek Chair in Mathematics, the Sciences, & Technology. During the grant year, Brad worked with collaborators to publish 1 research article, 1 review article, 1 book chapter, 1 curriculum resource (with Cathy Wheeler as coauthor), and 1 meeting review article. Even though no students were coauthors on these particular publications, the involvement of students in genomics and bioinformatics research was at the heart of most of these publications and the small contributions of many students were acknowledged. Brad also worked with collaborators and student coauthors on new manuscripts. Finally, Brad attended the 27th Annual Crown Gall Conference (oral presentation on research accomplishments involving undergraduates and high school students in genomics and bioinformatics), the 2007 Plant and Animal Genomes Conference (oral presentation on research accomplishments involving undergraduates and high school students in genomics and bioinformatics), the 2007 American Society of Microbiology Undergraduate Education Committee retreat (invited oral presentation on the need for national efforts to involve undergraduates in genomics and bioinformatics), the 2007 American Society of Microbiology Conference on Undergraduate Education (working to promote HHMI national effort on genomics and bioinformatics, as well as plan for sessions in the 2008 conference; recognized along with Cathy Wheeler with the Editors' Choice Award for 2006 for their MicrobeLibrary curriculum submission "Functional Genomics: Using Reverse Genetics to Test Bioinformatics Predictions"), the 2007 ASM/BIOQuest Workshop on Evolutionary Bioinformatics (session presenter), the 2007 American Society for Plant Biology Lab Leadership Conference (session presenter), and the 2007 Teagle Grant Workshop on "Teaching Big Science at Small Colleges: A Genomics Collaboration" (invited oral presentation on research accomplishments involving undergraduates and high school students in genomics and bioinformatics).

Stuart Gordon, Teaching/Research Postdoctoral Fellow, joined the Biology Department in March of 2007 with Brad Goodner and Cathy Wheeler serving as his mentors (his CV is attached at the end of this document). Stuart helped teach Microbiology in the spring of 2007, mentored several students in summer independent research, and worked with Brad and Cathy on course development for the upcoming academic year with a focus on research incorporated into the lab component of courses. In addition, Stuart worked with Brad to establish his own research program which will focus on using genomics and bioinformatics to better understand the biology of a plant pathogen, *Acidovorax avenae* subspecies *avenae*, that is of concern to maize and millet production in some parts of Africa. Stuart attended the Plant Associated Microbe Gene Ontology Workshop at the Virginia Bioinformatics Institute and brought back ideas for improving undergraduate bioinformatics education.

Michael Gumert, Teaching/Research Postdoctoral Fellow, joined the Biology and Psychology Departments in August of 2006 with Kim Phillips serving as his mentor. Michael worked with Kim Phillips and Courtney Buzzell to teach a new Behavioral Endocrinology course in the spring of 2007, and he mentored several students in independent research during the academic year supported by supplies purchased with grant funds. Michael attended the 2007 American Society of Primatology Conference where he organized and chaired a special workshop on his study organism, the long-tailed macaque. During the summer of 2007, Michael was offered and he accepted a faculty position at Nanyang Technological University in Singapore, which is much closer to his longterm study population of long-tailed macaques and to his wife's family. We wish Michael all the best for the future. Kim Phillips is currently trying to fill the remaining year of the vacant Teaching/Research Postdoctoral Fellow position.

Prudy Hall, Professor Emerita of Biology and Chemistry, incorporated some new bioinformatics and molecular biology research into the lab portion of her Plant Physiology course.

Sandy Madar, Associate Professor of Biology and Assistant Academic Dean of the College, mentored one student in grant-supported independent research during the summer (supported by research supplies purchased with grant funds) and published a research article with 4 student coauthors supported previously by summer research stipends from the grant.

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 the grant year, Jody incorporated research into the lab component of three different biochemistry courses and mentored 3 students in independent summer research, all supported by supplies purchased with grant funds.

Kim Phillips, Associate Professor of Biology and Psychology, mentored 4 students in grant-supported independent research during the academic year and 3 students during the summer (supported by research supply funds) and published an article with 1 student coauthor supported previously by a summer research stipend. In addition, Kim worked with Michael Gumert, Teaching/Research Postdoctoral Fellow, and Courtney Buzzell, Research Teaching Associate of Biology & Psychology, to teach Behavioral Endocrinology for the first time with research incorporated into the laboratory portion of the course. Kim attended the 2006 Society for Neuroscience meeting along with one student who gave a poster presentation, and the 2007 Midwest Primate Interest Group Meeting with two students who gave poster presentations.

Willa Schrlau (formerly Willa Ewing), Research Teaching Associate of Biology, worked with several faculty members in the Biology Department to revise the introductory biology two course sequence. Specifically, Willa was in charge of revising the lab components of both courses, with an emphasis on more quantitative reasoning and incorporation of research into the lab. Willa also attended the 2007 Association for Biology Laboratory Educators Conference (partially supported by some faculty development funds).

Oberta (Obie) Slotterbeck, Professor of Computer Science, has worked with her colleague Ellen Walker to develop their own brand of research-within-courses as part of the computer science curriculum in a very small department. Their Independent Research Components (IRCs) are very well respected on our campus and beyond. During the grant period, Obie and Ellen published an article on the use of IRCs in courses and she incorporated research into the Computer Simulation course supported by software purchased specifically for this course and a large server cluster purchased to serve the computational needs across the natural sciences (both purchases supported by grant funds).

Mark Taylor, Associate Professor of Physics, mentored two students in independent research supported by a large server cluster purchased with grant funds to serve the computational needs across the natural sciences. This work resulted in two published research articles with the students as coauthors.

Ellen Walker, Professor of Computer Science, has worked with her colleague Obie Slotterbeck to develop their own brand of research-within-courses as part of the computer science curriculum in a very small department. Their Independent Research Components (IRCs) are very well respected on our campus and beyond. During the grant period, Obie and Ellen

published an article on the use of IRCs in courses. Ellen also worked with Brad Goodner on plans to team teach bioinformatics in the spring of 2008.

Cathy Wheeler, Research Teaching Associate of Biology, worked with Brad Goodner to incorporate research into the Molecular & Cellular Biology, Genetics, and Microbiology courses (supported by course research supply funds) and to mentor students in independent research. Working with Brad, Cathy was the lead in running an outreach research collaboration with 3 area high schools during the academic year (support by outreach funds), a summer teachers workshop (supported by outreach funds), and 2 sessions of the Hiram Genomics Academy for high school students during the summer (both sessions supported by outreach funds). Cathy attended the 2007 American Society of Microbiology Conference on Undergraduate Education in order to promote genomics and bioinformatics education and gather information to help establish a new medical microbiology course for the new nursing program at Hiram College starting in the fall of 2007 (partially supported by some faculty development funds). At the conference, Cathy and Brad were recognized with the Editors' Choice Award for 2006 for their MicrobeLibrary curriculum submission "Functional Genomics: Using Reverse Genetics to Test Bioinformatics Predictions".

One of the challenges/opportunities we now face are how best to work with our new Teaching/Research Postdoctoral Fellows. We acknowledge that these young scientists have to get their own research programs up and going, but we can offer them an environment where they can make their research happen with undergraduates as the main workforce. This will be crucial if these future faculty members are aiming to move to PUIs and we have certainly advertised the positions as such. Michael Gumert left his position early only because a faculty position near his study site and some family members became available. He certainly benefited from having had some teaching experience in his one year at Hiram College. Stuart Gordon is well positioned to establish his own research program over the next year and develop an extensive teaching resume in the area of genetics, molecular biology, genomics, bioinformatics, and microbiology.

The other major challenge/opportunity is how best to enlarge the scope of our impact across the natural sciences. While some faculty members have willingly joined the grant team, many others in the current faculty will take more time to move in our direction. However, new faculty can be brought in with certain expectations and that is the most exciting development we can report. The growing importance of the integrated research-teaching model at Hiram College directly impacted the hiring of 5 new faculty members in the natural sciences. Positions in animal behavior/physiology, biochemistry, conservation biology, invertebrate ecology, 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.

HHMI Annual Progress Report

Grant Number: 52005125

Award Year: 2004

Institution name: Hiram College

Report Period: 09/01/2006 - 08/31/2007

Program Director: Brad Goodner

Curriculum Development

The historical view of research is that of an independent endeavor by an expert and yet all around us, scientific research demands teamwork and being open to new ways of thinking. 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 3, 10 faculty, staff, and postdocs (Eric Bauer, Courtney Buzzell, Colleen Fried, Brad Goodner, Stuart Gordon, Michael Gumert, Prudy Hall, Obie Slotterbeck, Kimberly Phillips, Cathy Wheeler) incorporated research projects into a total of 10 courses (Animal Physiology, Behavioral Endocrinology, Biochemical Research Methods, Computer Simulation, Genetics, Intermediate Biochemistry, Intermediate Organic Chemistry, Introductory Biochemistry, Microbiology, and Molecular & Cellular Biology) at Hiram College. These courses exposed 107 different students to research for a total of 226 experiences. Each research-within-course project is described below.

The newest course to incorporate novel research within the lab component was Behavioral Endocrinology, taught by Kim Phillips, Michael Gumert, and Courtney Buzzell. In the spring of 2007, 14 students worked for several weeks in small groups on independent research projects investigating various aspects of hormones and behavior. The projects ranged from cortisol responses and short-term memory in pregnant/lactating women v. non-pregnant women, behavioral and endocrine responses to novel foods in capuchins, and the impact of hormones and hormone antagonists on the behavior of insects. Some of these projects may be expanded upon in the next iteration of the course.

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. The project is currently in the annotation phase, where undergraduates can serve as the first layer of human experts to interpret what the genome is telling us. In the Fall 2006 semester, 46 students in the Molecular & Cellular Biology course were assigned in teams of 3-5 to specific amino acid biosynthesis, nucleotide biosynthesis, vitamin and cofactor biosynthesis, and central metabolism pathways. Each team had to learn their pathway – the number of steps involved, the number of proteins involved, and any alternative steps seen in certain bacteria. Then, each team had to reconstruct the pathway using the first-pass automated genome sequence annotation, trying to align each pathway step with the genes encoding the proper enzymes. Any cases of potential functional redundancy were noted, as were any steps for which no annotated gene was found. Given the prototrophy of *A. vinelandii*, missing steps of these pathways indicate either a misannotated gene or a novel gene encoding the enzymatic function of interest. Students learned to use BLAST to

discriminate between these two possible explanations. In the Spring 2007 semester, 50 students in the Genetics course were assigned in teams of 2-4 to continue a systems-level annotation by expanding both the number of pathways/processes analyzed and the breadth and depth of the analyses. Each team 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 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. vinelandii*. Such findings provide the initial evidence for horizontal gene transfer events. A great example of the surprises that come out of such deep analysis comes from the team assigned oxidative phosphorylation. This process seems rather mundane – simply accounting for all the cytochromes and ATP synthase components known to be involved in aerobic respiration. The students found all the usual players, including all the ATP synthase components grouped together in an apparent operon with clear orthologs in close relatives from the genus *Pseudomonas*. However, the students also found a second operon encoding a 2nd different set of ATP synthase subunits and this group had a different gene order and were more closely related to a different group of bacteria – Burkholderia and its relatives in the beta-Proteobacteria. This is a clear example of a horizontal gene transfer event and a small group of undergraduates are the experts!

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. Hiram College is part of a collaboration (with Arizona State University, Seattle Pacific University, Syracuse University and Virginia Tech University) to start a functional genomics effort to follow up on the *Agrobacterium* Genome Project (Brad Goodner, Co-PI, plus Cathy Wheeler and Stuart Gordon) using both reverse and forward genetics. For the 5th year in a row, 46 students in Molecular & Cellular Biology used reverse genetics over approximately 8 weeks of lab time to knock out specific genes in the previously sequenced *A. tumefaciens* C58 genome and then design experiments to test the putative function of those genes. Following up on the work of last year, each team of 2-3 students was assigned a gene encoding a two-component regulatory system response regulator subunit. The *A. tumefaciens* C58 genome encodes potentially 55 such response regulators and the Fall 2005 class knocked out 11 of those genes. The Fall 2006 class knocked out another 16 genes. Over the course of the semester, each team amplified a portion of their assigned gene by PCR and cloned it, and used their cloned sequence to disrupt the wildtype gene in *A. tumefaciens*. Making use of a microtiter plate reader purchased with grant funds, each team tested 24 of the two-component system mutants under a range of one environmental variable (e.g., pH). Most of the mutants showed a complex response to changes in growth conditions, but four of the mutants showed a consistent growth defect under any conditions that included low pH. All of these mutants will be further characterized in future courses.

Students in the Spring 2007 Genetics course used a forward genetics approach to connect genes to phenotypes in another *Agrobacterium* strain, *A. rhizogenes* A4, that is currently being sequenced. 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 3000 mutants. Genomic DNA was isolated and the site of transposon insertion cloned out for a few dozen potential mutants of interest and the DNA sequence of the transposon insertion site was obtained for 17 mutants. Genes involved in the synthesis of purine and pyrimidine nucleotides, histidine, leucine, glutamate, tryptophan, and arginine were identified.

Fifteen students in the Spring 2007 Microbiology course, working with Brad Goodner, Cathy Wheeler, and Stuart Gordon, 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 leaf surfaces from a variety of spring woodland wildflowers such as trout lily, spring beauty, toothwort, and ramps. Among culturable isolates, strains of *Bacillus*, *Pseudomonas*, and *Duganella* dominated. However, culture-independent methods showed a greater diversity especially among Gram- with many members of the gamma-Proteobacteria besides *Pseudomonas* and several alpha-Proteobacteria represented including *Agrobacterium*. Not only is this the way the science has moved, but culture-independent methods provide us with more routes for outreach to high schools at a lower cost and fewer headaches in terms of biological waste disposal, etc.

Eric Bauer and Courtney Buzzell redesigned the lab component of Animal Physiology (Spring 2007; 15 students) in order to make extensive use of new electrophysiology equipment (iWorx; purchased in part with grant funds) to study the neuromuscular system of frogs. Teams of students designed and performed independent projects on various aspects of neuromuscular physiology (e.g., ionic basis of synapses, pharmacology of synaptic transmission, and role of calcium in muscle temporal integration) during the second half of the semester. In the final lecture session, each team presented their findings to the rest of the class.

Prudy Hall has retired, but that hasn't stopped her from teaching or from learning new things and bringing them into her courses. Prudy has a long-term interest in the interaction of parasitic plants and their host plants, especially in terms of nitrogen metabolism. Prudy challenged her class to answer a novel question – do parasitic plants have the genetic capability to use nitrate, a common transport form of nitrogen in plants, as a nitrogen source. Specifically, ten students in the Spring 2007 Plant Physiology class tested plant extracts for NR activity using a simple colorimetric assay and used degenerate PCR to determine the presence or absence of a gene sequence encoding nitrate reductase (NR) in 2 species of parasitic plants. Students obtained genomic DNA from 2 parasitic plants (Beech Drops and Squaw Corn) and from 3 control plant species known to have NR genes (corn, soybean, and clover). Primers internal to well characterized NR sequences were selected and PCR was used to amplify the resulting sequences. Bands of known sizes were extracted from agarose gels and were sent off campus for sequencing. Bands of two predicted sizes were observed from samples of the two parasitic plants. The sequence derived from one band of both parasitic plants had good correspondence to the soybean NR. More interestingly, the sequence appears to correspond to the Hinge 1 region of NR that contains a binding site for regulatory proteins and a key serine residue that is subject to phosphorylation. One species of parasitic plant was notable in that a 4 amino acid deletion occurred in a closely adjacent region based on translation of the sequenced DNA sample. It is not known whether this deletion impacts enzymatic activity, but it is interesting to note that the students were unable to demonstrate NR activity in any extracts from the parasitic plants.

Jody Modarelli was hired this spring into a tenure-track position in Biochemistry, following in the footsteps of Prudy Hall. During most of grant year 3, however, she was a visiting adjunct

professor, yet she was willing to try the research-within-courses approach. In the Introductory Biochemistry course, in collaboration with Brad Goodner, 17 students spent a few weeks trying to overexpress a particular protein from *A. tumefaciens* C58 in *E. coli*. This particular protein is annotated as a sucrose hydrolase, but mutant analysis suggests it might have a different activity that might be related to mannosucrose production. Plasmid constructs were made with the intent of allowing the sucrose hydrolase gene to be expressed in a regulated manner. The protein product is a fusion protein consisting of the sucrose hydrolase protein hooked to a small domain acting as an affinity peptide. In this experiment, three different versions of the protein were expressed in *E. coli*: one full length version (93.1 kD), and two shorter versions (60.1 kD and 54.4 kD). The students attempted to solubilize proteins and to detect them by 1-D gel electrophoresis, but were unsuccessful. This work continues in the current iteration of the course. The Intermediate Biochemistry and Biochemical Research Methods classes, with 11 and 4 students respectively, investigated differences in the lipid content in urine between males and females. The fatty acids in urine were extracted using organic solvents and detected by a combination of liquid chromatography mass spectrometry (LC-MS). The fatty acids were also esterified to their methyl esters and detected by gas chromatography (GC-MS). Male urine samples showed differences in fatty acid constituents. This experiment is a precursor to a study looking at the urine in patients with renal cell carcinoma and comparing them to healthy controls.

Students in the Intermediate Organic Chemistry course continued to expand the use of the microwave accelerator labstation purchased with grant funds. In the spring of 2007, 33 students used the microwave accelerator to cut down the time on classic reactions such as the Diels-Alder reaction, which typically takes temperatures of 200 C and extended reaction times, but also to more complex organic syntheses as well. The ultimate goal is now within reach - to have students carry out organic synthesis projects with only a starting point defined. Each student would identify three appropriate target compounds, design appropriate synthesis strategies, put their ideas to the test, and finally isolate and characterize their product. Since many of the target compounds chosen lie outside the typical indices of organic compounds, students would have to use related compounds to estimate chemical characteristics (e.g., boiling point) of their target compound.

Research-within-courses is known by another name in the computer science curriculum at Hiram College - Independent Research Components (IRCs). Each student in the major must complete a certain number of IRC projects during their career by tagging on the extra project to various courses throughout the major. Computer science, beyond its impact on bioinformatics, was originally outside the scope of this grant proposal. However, the impact of the large server cluster purchased with grant funds in year 1 was highly underestimated. Obie Slotterbeck, Professor of Computer Science, challenged her 12 students in the Computer Simulation course to think big in using the parallel processing of the server cluster and when they responded with some complex IRC proposals, such as modeling how rapidly mutation-selection schemes can change a DNA sequence, she asked for help in obtaining a more sophisticated simulation software package (Arena). Given that these students are doing exactly what this grant intended - learning by doing original research within a course- it was an easy choice to support the purchase of the software with grant funds. Future courses will expand the use of the software.

While we are always happy with impacting a large number of undergraduates, the most heartening aspect of grant year 3 was the large increase in the number of faculty who incorporated research into their courses with grant support. This trend looks to be on a stable upward trajectory because many additional faculty have signed on to this approach for the future.

HHMI Annual Progress Report

Grant Number: 52005125

Award Year: 2004

Institution name: Hiram College

Report Period: 09/01/2006 - 08/31/2007

Program Director: Brad Goodner

Precollege and other Outreach Programs

In grant year 3, Brad Goodner and Cathy Wheeler 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.

During the academic year, Brad and Cathy continued outreach collaborations with biology classes at three area high schools - The Andrews School (private, suburban, all female), Cleveland Benedictine (private, urban, all male), and Rootstown (public, rural). During the academic year, 5 students from The Andrews School and 75 students from Rootstown High School generated and screened over 4000 transposon insertion mutants for the *Agrobacterium tumefaciens* C58 genome project. Over 40 mutants with auxotrophic and pH-sensitive phenotypes were identified. Over one week in December, 2006, Ms. Stephanie Lammlein and her Rootstown students brought their mutants to Hiram College in three waves. Brad Goodner and Cathy Wheeler helped them isolate genomic DNA and begin to learn how to interpret DNA sequence information. Back at their home school, the students cloned out their transposon insertion sites and they were sent off for sequencing. Brad Goodner gave the students another lesson in bioinformatics and the students spent a couple of weeks connecting the identification of their mutant genes back to the altered phenotypes of their mutants, linking genes to basic cellular functions. All along the way, Ms. Lammlein led her students through a book reading and discussions of the science behind genetically modified crops, where *Agrobacterium* is used as a gene delivery agent, and the hopes and concerns that come with the use of those crops. The big finale came on May 23rd, 2006, when the students at Rootstown High School presented posters on their year-long projects to their families and other members of the community. The turnout surprised everyone and it was clear that the students had experienced a lot of fun, a healthy dose of integrated learning about basic biology and biological applications to agriculture, and a chance to see themselves as young scientists who can contribute to novel research as part of their learning.

Diana McBeath and her students at Cleveland Benedictine High School have worked with us for the past six years on a variety of genome projects, but this year Ms. McBeath wanted to try something different. She takes her students every fall to the Cuyahoga Valley National Park for a day of ecological sampling. This past fall, she asked her students a new question during that outing – “do you ever wonder what grows on the surface of a leaf, underneath pebbles at the bottom of a stream, or on the wall of a cave?” Ms. McBeath and her students brought environmental samples from the Cuyahoga Valley National Park back to the lab where they plated out dilutions on different microbiological plate media. They learned sterile technique and eventually were able to get many bacterial isolates into pure culture. The students then used a combination of classic culture-based identification methods and modern PCR-based techniques to learn more about their strains. Amplified 16S rRNA genes were sequenced and compared to publicly available databases. Strains from the genera *Acinetobacter*, *Aeromonas*, *Bacillus*,

Exiguobacterium, *Flavimonas*, *Janthinobacterium*, *Kocuria*, *Pseudomonas*, and *Serratia* were confirmed.

During the summer, Brad and Cathy ran a teacher workshop followed closely by two sessions of the Hiram Genomics Academy, all supported by grant funds and by assistance from the college administration. Five teachers from across northeast Ohio, representing a wide range of public and private high schools, participated in a 3-day workshop where they were introduced to several possible projects that they could take back to their home schools. The teachers then split into two groups and each group helped Brad and Cathy run a 2½-day session of the Hiram Genomics Academy. In this way, the teachers could see how students would react to concepts and to various lab skills as well as try out their own teaching methods in a stress-free environment. A total of 37 high school students from across Ohio, Pennsylvania, Michigan, and Indiana participated in functional genomics research as part of the collaborative *Agrobacterium* genome project mentioned in an earlier section. In each session 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 transposon insertion mutants of *Agrobacterium tumefaciens* C58 for 10 different phenotypes, and the isolation of genomic DNA and cloning out of the transposon insertion site for 15 mutants with auxotrophic, pH-sensitive, and biofilm-defective phenotypes. Several of the mutants first identified by high school students have been the focus of further studies by both high school and undergraduate students.

The final outreach activities were aimed at our colleagues working in undergraduate education. Brad made six presentations to a wide variety of audiences* on the role that genomics and bioinformatics research can play in undergraduate and high school education. He also published several articles dealing with this same issue and he co-wrote a HHMI Program Directors' minigrant proposal (with Sally Elgin of Washington University in St. Louis) that was funded to support discussions and actions to lower the barriers to more widespread involvement of undergraduates in such research. He assisted, as called upon, in the formative stages of two national efforts, one by HHMI (SEA led by Tuajuanda Jordan) and the other by DOE-JGI (the education side of GEBA led by Cheryl Kerfeld). Finally, Brad and Cathy shared their expertise, strains, and curriculum resources with colleagues across the country and in Canada, as well as through a published curriculum resource in MicrobeLibrary that was selected for the Editors' Choice award for 2006.

* Plant and Animal Genomes Conference, ASM Undergraduate Education Committee retreat, ASM/BIOQuest Workshop on Evolutionary Bioinformatics, American Society for Plant Biology Lab Leadership Conference, & Teagle Grant Workshop on "Teaching Big Science at Small Colleges: A Genomics Collaboration"

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