

Major Outcomes

Undergraduate Research Within Courses

A total of 108 undergraduate students participated in original research projects as part of the laboratory components of 7 different courses at Hiram College (2 iterations of Molecular & Cellular Biology, 2 iterations of Genetics, Biochemistry I, Biochemistry II, and Microbiology). As one example, 49 students in 3 courses participated in sequence annotation of the *Chromohalobacter salexigens* genome and presented their results in a symposium on October 8th, 2004. Students in the Molecular & Cellular Biology course reconstructed metabolic pathways from the *C. salexigens* genome annotation and compared them with related bacteria. Genetics students compared *C. salexigens* and its close relatives in terms of gene order and possible regulatory circuits for metabolic pathways. Students in the Biochemistry course compared the isoelectric points for predicted protein sequences across four large cellular localization groups (cytoplasmic, plasma membrane, periplasmic, and outer membrane) to test the hypothesis that extracellular proteins of halophilic organisms have adapted to a more negative overall charge that prevents their precipitation under high salt conditions. As a second example, 48 Molecular & Cellular Biology students conducted functional genomics research on the soil bacterium and plant pathogen *Agrobacterium tumefaciens*. Teams of 2-3 students were assigned a gene with a predicted cellular function based on the *A. tumefaciens* C58 genome sequence. Each team amplified a portion of their assigned gene by PCR and cloned it, used their cloned sequence to disrupt the wildtype gene in *A. tumefaciens*, designed and carried out 1-2 experiments to test the putative function of their assigned gene, and presented their results.

Sharing Research Output with Others

We shared our successes with others in two ways. First, based on research done within 7 courses and through independent projects mentored by 5 Hiram College faculty, 18 undergraduate students presented their work through 9 poster and 6 oral presentations at 5 scientific conferences (25th & 26th Annual Crown Gall Conferences, 2005 Ohio Academy of Science Annual Meeting, 2005 *Xenorhabdus* Genome Consortium Meeting, & Evolution Of Aquatic Tetrapods 4th Triannual Convention) and through 1 published book chapter. Second, 2 faculty members shared their experiences of incorporating research within courses through the publication of one laboratory project in the Web-based ASM Microbe Library and through 2 conference presentations (2005 ASM Conference on Undergraduate Education and 2005 ASM General Meeting).

Research Outreach to High School Students

Our outreach efforts focus on involvement of high school students in research collaborations with students and faculty from Hiram College. During the 2004-2005 academic year, 10 students from Cleveland Benedictine High School participated in transposon mutagenesis as part of the *Xenorhabdus nematophila* genome project. During the summer of 2005, 7 high school students from different schools participated in a two week summer research camp where they conducted transposon mutagenesis, mutant characterization, and physical mapping of mutations as part of the *Azotobacter vinelandii* genome project. Also during the summer of 2005, 3 teachers from different high schools spent 2 weeks learning transposon mutagenesis, recombinant DNA cloning, and bacterial genetics in preparation for collaborative research projects in the upcoming 2005-2006 academic year.

Student Research and Broadening Access to Science

During the first year of our HHMI grant, 35 undergraduates at Hiram College took part in research through independent projects mentored by a total of 6 faculty members associated with the grant. This research effort was separate from student involvement in research within courses (described in the Curriculum, Equipment, & Laboratory Development section). Of these 35 students, 18 were supported by summer research stipends (14 full-time & 4 half-time), 8 were supported by academic year stipends, and all of them were supported by research supplies purchased with grant funds. Based on the independent projects described in this section and on research done within 7 courses (described in the Curriculum, Equipment, & Laboratory Development section), 18 undergraduate students presented their work through 9 poster and 6 oral presentations at 5 scientific conferences (25th & 26th Annual Crown Gall Conferences, 2005 Ohio Academy of Science Annual Meeting, 2005 *Xenorhabdus* Genome Consortium Meeting, & Evolution Of Aquatic Tetrapods 4th Triannual Convention) and through 1 published book chapter. The following paragraphs describe each research project, the students and faculty involved, and any outcomes so far.

Handedness and Brain Asymmetries

Student: Ashley Cipollone

Mentors: Kim Phillips & Courtney Buzzell

Anatomical asymmetries of the human brain are thought to underlie the expression of lateralization of functions such as hand preference and hemispheric language dominance. Individual capuchin monkeys (*Cebus apella*) demonstrate laterality of function in the areas of hand grasping and object manipulation; however, they do not exhibit the strong-population-wide tendency to right-handedness found in humans. Therefore, this species provides a unique opportunity to investigate the relationship between intraspecific variation in handedness and brain lateralization. For this project we obtained structural magnetic resonance images (MRI) from nine adult and subadult capuchin monkeys to correlate behavioral lateralization to asymmetries of specific brain areas. We also collected behavioral data on hand grasping and object manipulation for each subject. To date, we have analyzed handedness and structural asymmetries in our male capuchins ($n = 7$). We are currently expanding our sample size by adding two additional adult females, housed at a nearby research institution (NEOUCOM). As an MRI database of capuchin brains does not exist, these scans will be a rich resource for investigations. We have incorporated these scans into our Neuroscience lab course, allowing students to develop and test questions relevant to the evolution of brain and behavior.

Outcome: The findings from this research has recently been accepted for publication in the journal *Behavioral Neuroscience* (co-authored with a colleague at Kent State University, Chet Sherwood), but will be published in grant year 2.

Color Vision and Foraging Decisions in *Cebus paella*

Students: Betsy Shaw & Mike Sliter

Mentors: Kim Phillips & Courtney Buzzell

Color vision among primates is hypothesized to have evolved as an adaptation for detecting ripe fruits or young leaves against a background of mature leaves. Most New World monkeys, including *Cebus*, have color vision polymorphisms, with individuals having either dichromatic or trichromatic color vision. All male capuchins are dichromats and have a form of

color vision that resembles a form of color blindness in humans. About 2/3 of female capuchins have trichromatic vision; the remaining 1/3 are dichromats. A single gene on the X chromosome codes for the opsin proteins that code for photopigment. Three alleles of the opsin gene exist, each of which specifies one of the three medium-long length wavelength photopigments. All males and females that are homozygous for the opsin gene are dichromats. Females that are heterozygous for this gene are trichromats. The current study consists of two main parts: 1. to determine the genotype, and thus visual phenotype, of all capuchin subjects, and 2. to design an apparatus to determine whether or not trichromats have a foraging advantage over dichromats. The apparatus will present a foraging task consisting of stimuli that resemble the shape, size and color of actual leaves and fruit encountered in the wild. To determine the visual phenotype of the Hiram College capuchins, individual blood samples from all subjects were obtained during our annual animal health check. All extraction and amplification procedures, and scoring of genotypes will be conducted by Betsy Shaw, under the guidance of Dr. Brad Goodner of the Hiram Genomics Lab. Betsy also worked this summer on extracting DNA from fecal samples. Inconclusive results led us to switch to the use of blood as a source for DNA. On the behavioral testing front, we are currently in the stages of designing a foraging apparatus. Reflectance data from *Spondias mombin* (a fruit favored by *Cebus apella*) were collected in Nicaragua this summer using a portable USB2000 spectrometer and tungsten-halogen light source (Ocean Optics, Inc.). From these data, we are creating artificial leaves and unripe, mid-ripe, and ripe fruits. Small colored boxes (our "fruits"), spectrally similar to unripe, mid-ripe, and ripe *Spondias* fruits, will be presented to a subject surrounded by a leafy background. Ripe fruit boxes will have the greatest reward inside, mid-ripe boxes a small reward, and unripe boxes no reward. Subjects will be tested for their ability to select ripe fruits against the camouflaged leafy background. If the subject is able to differentiate between unripe, mid-ripe, and ripe colors, it is expected that the selection of fruit boxes should be nonrandom.

Sensory Cues in Food Selection

Student: Ashley Cipollone

Mentors: Kim Phillips & Courtney Buzzell

Capuchins employ tapping as a means of gathering information about the location of an invertebrate embedded in bamboo or wood (Phillips et al., 2003; Phillips et al., in press). Field observations suggest that capuchins also employ tapping while foraging on hard nuts and fruits, as a means of gathering information about the foods. The purpose of this study is to examine the sensory cues that capuchins use in foraging. The study (modified from Visalberghi & Néel, 2003) tests whether capuchins can discriminate between full and modified walnuts on the basis of weight, sound, and vibrational cues. For all trials subjects are presented with two walnuts for investigation: one full, one modified. Nuts were modified in four ways: phase 1 (full, empty): differ in weight, sound, vibration; phase 2 (full, paper-filled): differ in weight, vibration; phase 3 (full, steel nut-filled): differ in sound, vibration; and phase 4 (full, paper and steel nut – filled): differ in vibration. To date, all subjects (n = 6) have completed Phase 1. Five out of six subjects directed significantly more investigative behavior (e.g., tap, sniff, bang) at the full versus empty walnut, suggesting that one or all of these cues are used in foraging. The completion of all four phases will allow us to assess which cues, or combination of cues, are necessary for capuchins in making foraging decisions.

Socioendocrinology

Student: Karya Ottey

Mentors: Kim Phillips & Courtney Buzzell

Prior to this year, our lab has been involved in several behavioral endocrinology studies; however, we have needed to send our samples away for analysis. The addition of enzyme immunoassay equipment (as part of the current HHMI grant), has provided the opportunity to focus such research here at our institution. This will be an invaluable tool for both our research and the undergraduate lab experience. We have started several projects related to this area of study. We currently are collecting long-term data on social interactions among our animals, and are specifically interested in male-male relationships. During this period we have been collecting fecal samples to provide a physiological component (e.g., testosterone) to the study. This summer we have begun to process our fecal samples for extraction and have started to develop our extraction protocol. Another project underway is the study of the physiological mechanisms involved in altruistic behavior (e.g., cooperation in primates). This summer we have been working with our animals to provide saliva samples (see training section below). Saliva samples will be analyzed for prolactin, cortisol, and salivary immunoglobulin A, all of which have been implicated in social bond formation, health, and/or well-being. As our extraction and assay protocols are developed, they will be integrated into laboratory exercises in the new Behavioral Endocrinology course and our existing Neuroscience course. Currently, we are working on integrating a lab for analyzing the human stress hormone cortisol. We plan to use this as a starting point for student-led investigations into hormone-behavior interactions in capuchins.

Testing Food Preferences with a Tool-Use Task

Student: Nick Morgan

Mentors: Kim Phillips & Courtney Buzzell

The purpose of this study is to assess the effort capuchins are willing to exert in order to obtain desirable food items compared to less desirable food items. In captivity, capuchins demonstrate preferences for certain food items at daily feedings. The capuchins housed at Hiram College also show food preferences, but there are no quantitative data on food preferences for our animals. To study this, a task was designed that requires animals to exert significant effort to obtain food items. The task was made difficult by requiring the use of a tool to manipulate an out-of-reach food item. Capuchins are one of the few New World monkeys that are tool users, both in controlled environments and, in limited cases, in their natural habitats. Three phases have been created in order to train and test the capuchins on this task. The purpose of phase one is to habituate subjects ($n = 7$) to the tool and testing apparatus and to train subjects to use the tool effectively. In phase two, subjects are trained to manipulate their tool around barriers to obtain a food reward. In Phase three subjects are tested on the task with preferred and non-preferred foods. This summer's (2005) research has been focused on phase one, training and habituation. Training the animals to use tools effectively is a timely process, as subjects must learn to manipulate the tool successfully through trial and error learning. For training, phase one consisted of six sessions, with each session increasing in the complexity of tool manipulation required to attain success. In each session the subjects were presented with a tool and an out-of reach food reward. The tool and reward were oriented differently depending on the session, and required more complex manipulations from session one to six. All subjects successfully completed phase one of training this summer, and are moving on to phase two. Phase two is similar to phase one, but there is an introduction of barriers to make the food reward difficult to obtain. Phase three begins the process of looking at food preference, where varying foods are introduced and level of effort assessed.

Training Using Positive Reinforcement

Student: Betsy Shaw

Mentors: Kim Phillips & Courtney Buzzell

Two training programs were initiated this summer. The first program was designed to facilitate reliable saliva collection from the animals. Several hormones of interest in our lab (e.g., cortisol) can be directly extracted and assayed from saliva. Saliva can be collected non-invasively, by having the animal chew on a cotton rope (similar to how it is collected in humans). The purpose of training was to get animals to chew for at least 30 seconds, giving us consistent samples. The purpose of the second program was to introduce target training to the animals. Subjects were trained to respond to a specific symbol (target), unique for each subject. Such training can help to facilitate cooperation of animals in research and husbandry procedures and provide enrichment.

Further Links Between Sugar Metabolism & Osmotic Tolerance in *Agrobacterium*

Students: Mandy Reed, Virginia Mateo, Ben Shelton, Jessica Edwards, Frank Arnold, & Josh Collins

Mentors: Brad Goodner & Cathy Wheeler

Previous work in our lab has shown that while there is functional redundancy for sucrose catabolism in *A. tumefaciens* C58, the gene annotated as sucrose hydrolase (= AGR_C_1721 = Atu0944) is important for osmotic tolerance. Our current hypothesis is that sucrose hydrolase, unique to biovar 1 strains, actually catalyzes the synthesis of mannosucrose which is known to be accumulated under osmotic stress. We are continuing to test this hypothesis. To further explore the genetic basis for osmotic tolerance, we conducted a large scale screen for mutants of *A. tumefaciens* C58 (biovar 1) unable to grow either under high salt conditions or with low levels of sucrose as a sole carbon source. As we will show in this poster, most of the mutants characterized so far have substantiated the link between sugar metabolism and osmotic tolerance. Finally, we are continuing our efforts to characterize the basis of the 3-ketosucrose metabolic pathway in *A. tumefaciens* C58. We now have a few mutants that may allow us to dissect this pathway.

Outcome: Poster presentation by Mandy Reed at 26th Annual Crown Gall Conference, August 5-7, 2005 in Bloomington, Indiana.

Functional Differences Between the Two Aconitases of *A. tumefaciens* C58

Students: Terrence Johnson, DaJuan Whiteside, & Leora Livingston

Mentors: Brad Goodner & Cathy Wheeler

Aconitases are monomeric iron-sulfur proteins whose primary function is to catalyze the interconversion of citrate to isocitrate in the citric acid cycle. Most members of the Bacteria domain have two aconitases, AcnA and AcnB. According to a model developed in *E. coli* and *B. subtilis*, a second function of aconitases is to monitor oxidative stress and post-transcriptionally regulate the synthesis of additional aconitase protein as well as cellular processes such as motility. In *E. coli*, AcnA is induced during periods of stress while AcnB is the major enzyme involved in exponential growth. Mutations in *acnA* have no impact on motility, while mutations in *acnB* decrease motility. Unlike the *E. coli* model, the sequenced members of the alpha-Proteobacteria have only a *acnA* gene with rare exceptions such as *A. tumefaciens* C58 which has both *acnA* and *acnB*. To analyze the role of the two aconitases in *A. tumefaciens* C58, we generated mutations in each gene (AGR_C_4866 = *acnA*; AGR_L_294 = *acnB*). Contrary to the *E. coli* model, the *acnA*⁻ mutant is an auxotroph on minimal media and it shows a very

hypermotile phenotype on rich medium. The *acnB*⁻ mutant shows only a mild hypomotile phenotype. The hypermotility of the *acnA*⁻ mutant appears to be due to hyperflagellation, both in number and in length. However, the hypermotility does not substantially impact virulence, due to the repression of motility by low pH conditions.

Outcome: Poster presentation by Terrence Johnson & DaJuan Whiteside at 26th Annual Crown Gall Conference, August 5-7, 2005 in Bloomington, Indiana. Winner of the Midwest Scientific Award for Best Undergraduate Poster.

Unearthing the Linearization of Chromosome II in the *Agrobacterium* Biovar 1 Clade

Students: Erin Henry, Allison Sabo, & Erin Telepak

Mentors: Brad Goodner & Cathy Wheeler

Past genetic/physical mapping has shown that *Agrobacterium* biovar 1 and biovar 3 strains harbor two chromosomes, and the complete sequence of *A. tumefaciens* C58 provided strong evidence for the plasmid origin of chromosome II. However, the chromosome II of biovar 1 differs dramatically in its topology (linear with hairpin loop ends) from that of its homolog in biovar 3 (circular). We have found that all biovar 1 strains tested so far and some closely related *Rhizobium* strains have both a linear chromosome II and the gene now known to encode the enzyme required for hairpin loop maintenance. The simplest hypothesis to explain the origin of the linearity of chromosome II in the biovar 1 clade and a system for its maintenance is one where both the hairpin loops and the protelomerase gene entered the genome at the same time. Our working model proposes that an illegitimate recombination occurred between the circular form of chromosome II and a linear phage genome. No such truly linear *Agrobacterium*-specific phage has ever been described in the literature, only lambdaoid-type phages with cohesive ends that form a circle in vivo. We are using two strategies to look for *Agrobacterium*-specific phages, with the hopes of finding phages with linear genomes. Our first strategy is a traditional phage search of plant tumor and soil samples using several *Agrobacterium* strains as hosts. We have not had a tremendous amount of success so far. Our second strategy is a PCR-based approach that should identify any biovar 1 strain or phage in an environmental sample that contains a protelomerase gene. After isolation of total DNA from samples, step one is a straightforward PCR using primers to amplify an internal portion of the protelomerase gene. For any samples with success in step one, total DNA is cut and circularized, then used for inverse PCR with primers reading out from near each end of the protelomerase gene. Successful amplification will yield the sequences surrounding the protelomerase gene. Knowledge of the gene neighborhood will help us dissect the evolutionary history of the protelomerase gene and may eventually yield a phage-like neighborhood consistent with our working model.

Outcome: Poster presentation by Allison Sabo & Erin Telepak at 26th Annual Crown Gall Conference, August 5-7, 2005 in Bloomington, Indiana.

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

Students: Nabil Abraham, Ian Bennett, Telisha Law, Frank Arnold, & Josh Collins

Mentors: Brad Goodner & Cathy Wheeler

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). We are currently focusing on the roles of the two different nitrate reductases in the *A. tumefaciens* C58 genome. Using gene disruption mutations in the genes encoding the large subunits of the NAS-type and NAP-

type nitrate reductases, we have found some evidence for these enzymes having overlapping functions. A much more controversial question is whether *Agrobacterium* can grow in the absence of any added nitrogen in the growth medium. This question of nitrogen fixation/nitrogen scavenging (we are unable to separate the two possible phenomena at this point) 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. We will present evidence that this is real growth is not due to nutrient carryover. Since the C58 genome clearly shows the absence of known nitrogenase subunits, the observed fixation/scavenging must be due to some novel biochemical reactions. We are currently using a mutant hunt approach to identify the genes required for these reactions.

Outcome: Poster presentation by Nabil Abraham & Ian Bennett at 26th Annual Crown Gall Conference, August 5-7, 2005 in Bloomington, Indiana.

Ongoing Collaborative Genome Projects Involving Hiram College

Students: Divya Balasubramanian, Adam Ewing, Dan Factor, Stephanie Larrick, Becky Roemer, Andee Wilson, Lindsey Wilson, & students in 2004-2005 Biochemistry & Genetics & Molecular and Cellular Biology courses

Mentors: Prudy Hall, Cathy Wheeler, & Brad Goodner

Since the publication of the *A. tumefaciens* C58 genome in late 2001, we have been privileged to participate in several other genome projects. Our contributions vary with each project depending on the needs of the project and how it fits in with courses at Hiram College, and range from the generation of physical and genetic maps of chromosomes and plasmids to gap closure to sequence annotation. In our first effort, two more strains of *Agrobacterium*, the grape pathogen *A. vitis* S4 and the well known biocontrol agent *A. radiobacter* K84 are being sequenced by the K84/S4 Genome Consortium and are now in the annotation phase. We confirmed the number and sizes of the genomic components of strain S4, used bioinformatics-based approaches to help with the assembly of both genomes, and are currently assisting in the annotation of both genomes. In our second effort, two species of *Xenorhabdus*, *X. bovienii* and *X. nematophila*, are being sequenced by the *Xenorhabdus* Genome Consortium. These bacteria pull off a Jeckyl and Hyde – they form mutualistic associations with certain nematodes and then together kill particular insect larvae to use as nutrient sources for rapid proliferation. The *X. bovienii* genome is in the annotation phase and *X. nematophila* is very close to closure of the genome sequence. Our main job here is annotation, although we have also helped with the assembly of both genomes. In our third effort, *Chromohalobacter salixegens* (formerly *Halomonas elongata*) is one of the most halotolerant members of the domain Bacteria. The DOE Joint Genome Institute provided draft sequence of its genome and we are now collaborating with Dr. Laszlo Csonka at Purdue University and the DOE-JGI to finish the genome and annotate it. We are providing a partial physical/genetic map of the genome, bioinformatics-based gap closure, and sequence annotation. In our fourth effort, *Azotobacter vinelandii* is a well known, free-living, aerobic nitrogen fixer. A draft of its genome has been done by DOE-JGI and we are now part of a recently funded *Azotobacter* Genome Consortium to finish and annotate the genome. In preparation for those efforts, we are currently starting a physical/genetic map of the genome.

Hiram College
Howard Hughes Medical Institute Grant
2004-2005 Accomplishments

Outcomes: (1) Poster presentation by Lindsey Wilson at 26th Annual Crown Gall Conference, August 5-7, 2005 in Bloomington, Indiana. (2) Published book chapter with Adam Ewing as an author (Csonka, L.N., K. O'Connor, F. Larimer, P. Richardson, A. Lapidus, A.D. Ewing, B.W. Goodner, & A. Oren, 2005. What we can deduce about metabolism in the moderate halophile *Chromohalobacter salexigens* from its genomic sequence. Chapter 18 in Adaptation To Life At High Salt Concentrations In Archaea, Bacteria, and Eukarya. Gunde-Cimerman, N., A. Oren, & A. Plemenita, eds. Springer-Dordrecht.). (3) Poster presentation by Adam Ewing at 2005 Ohio Academy of Science General Meeting in Bowling Green, Ohio. (4) Oral presentations by Dan Factor, Becky Roemer, Andee Wilson, Stephanie Larrick, and Lindsey Wilson at *Xenorhabdus* Genome Consortium Meeting, July 7-8, 2005 in St. Louis, Missouri.

***Agrobacterium* Functional Genomics Studies at Hiram College**

Students: Frank Arnold, Divya Balasubramanian, Josh Collins, Dan Factor, Stephanie Larrick, Chris Penton, Becky Roemer, Andee Wilson, Lindsey Wilson, Sarah Zilka, & students in 2002-2005 Molecular and Cellular Biology courses

Mentors: Prudy Hall, Cathy Wheeler, & Brad Goodner

Obtaining the complete genome sequence of any organism is rather like finding a hidden library from another civilization – you know there is important information inside but cannot make sense of all it right away. The genome sequence of *A. tumefaciens* C58 yielded many genes we completely expected, some genes that we are not too surprised to see, some surprising finds, and some enigmas/wild goose chases (only time and experiments will tell). Starting in the spring of 2002, both within courses and through independent projects, we have been experimentally testing the putative functions of several genes each year. The putative functions of most of the genes tested arose from the published sequence annotation, however a few of them came out of forward genetic mutant hunts, and one came out of the lack of bioinformatic support for an assayable function. In 2004-2005, we targeted the following genes for knockout and initial mutant characterization: xylulokinase, 2 alpha-galactosidases, beta-glucosidase, choline dehydrogenase, organic solvent tolerance protein, copper tolerance protein, 2 aconitases, 3 glutamate synthase large subunits, sulfite reductase flavoprotein subunit, and sulfite flavoprotein hemoprotein subunit.

Outcome: Poster presentation by Dan Factor at 26th Annual Crown Gall Conference, August 5-7, 2005 in Bloomington, Indiana.

Diversity of Responses to Selenite Among *Agrobacterium*

Students: Joshua Collins, Frank Arnold, Dan Ondrusek, Jennifer Leising, Chris Penton, & students in 2004 Microbiology course

Mentors: Brad Goodner & Cathy Wheeler

Recently, we have gone back to some old semi-selective media as part of biovar typing for various *Agrobacterium* projects. We noticed that older colonies of some strains acquired an orange pigmentation on Schroth and New/Kerr media, and one of the only common components is the antimicrobial sodium selenite that inhibits the growth of most Gram-negative bacteria at submillimolar concentrations. We now have evidence that this orange pigmentation is actually the reduction of selenite to the amorphous form of elemental selenium, one potential way to detoxify the oxidizing agent. Given that many other *Agrobacterium* strains grow on these same media without the pigmentation change suggests multiple detoxification and/or reduction pathways, consistent with what is known in the literature. A large-scale mutant hunt has

implicated roles for sulfite reductase, a thioredoxin reductase family member, a ArsR-type transcription factor, and another transcription factor (TspO) that might regulate stress responses. This work continues.

Outcome: Poster presentation by Frank Arnold at 25th Annual Crown Gall Conference, 2004, in Champaign-Urbana, Illinois.

Induction of a Nonribosomal Peptide/Polyketide Biosynthesis Gene Cluster

Students: Dan Ondrusek, Jennifer Leising, & students in 2004 Genetics & Microbiology classes
Mentors: Brad Goodner, Cathy Wheeler, & Colleen Fried):

Several years ago, we presented some initial bioinformatics and functional characterization of a large gene cluster in *A. tumefaciens* C58 that appears to encode a pathway for the synthesis of a hybrid nonribosomal peptide/polyketide (nrp/pks). We found no evidence that the gene cluster was expressed during a normal growth curve in rich or minimal media, or during starvation. Also, we found no clear phenotype for a mutant missing one of the enzymes in the pathway and so we put the project on the back burner at that point. This spring, as part of a new research project within our genetics course, we used a *lacZ* fusion in AGR_L_2329/Atu3672, a gene within the cluster that encodes a multidomain polyketide synthase, to monitor expression of the gene cluster in response to a variety of conditions. Acetosyringone, low pH, and iron starvation were a few of the treatments that failed to induce the gene fusion. The only condition that showed induction was addition of an aqueous extract from radish seeds or seedlings. Interestingly, these extracts also contain a bacteriostatic growth inhibitor. We went on to show that the inhibitor is sulforaphene, a known component of radish seeds and seedlings. However, in checking out all possible explanations for the induction, we eventually found that the beta-galactosidase activity was coming from the radish extract itself and not the *A. tumefaciens*! (During this time, another lab at University of Wisconsin showed that the nrp/pks gene cluster is involved in the synthesis of a unique siderophore.)

Outcome: Poster presentation by Dan Ondrusek at 25th Annual Crown Gall Conference, 2004, in Champaign-Urbana, Illinois.

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

Students: Noel-Marie Gray, Kim Kainec, Lucas Tomko, & Scott Wolfe
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. Although members of one late family of archaic whales have been described with pachyostosis considered skeletal ballast, with few exceptions, modern cetaceans exhibit osteoporosis and control buoyancy dynamically. The past fifteen years of paleontological effort has provided an unprecedented opportunity to examine the osteological transformation of whales as they make their transition to obligate aquatic lifestyle over a ten million year period. We analyzed the bone histology of five early cetacean families, including Pakicetidae, Ambulocetidae, Protocetidae, Remintonocetidae, and Basilosauridae. We then examined whether whales manifest their osteological specialization in the same manner as extant semi-aquatic and fully aquatic mammals. While few gross morphological adaptations related to swimming are seen in the earliest whales, histological analysis clearly shows extreme bone density that is connected to an aquatic specialization.

Outcome: Oral presentation by Noel-Marie Gray at Evolution Of Aquatic Tetrapods 4th Triannual Convention, May 16-20, 2005, in Akron, Ohio.

Significance Of Body Proportions In The Transition To Dorsoventral Undulatory Modes Of Swimming In Archaeocete Whales

Student: Amy Maas

Mentor: Sandy Madar

We expanded upon the past efforts of P.D. Gingerich to examine skeletal proportions and locomotor modes of modern semi-aquatic mammals, as a means of understanding locomotion in early whales. Utilizing the same taxonomic sample of modern semi-aquatic mammals as Gingerich, we have been able to expand the morphological scope of the data set to include eight additional metrics from the neck, sacrum and tail, using newly described fossil material from several archaeocete families. As modern cetacean locomotion is powered by muscular forces generated by the lumbar and caudal spinal segments, we seek to test the assertion that there were two stages in evolutionary transition to oscillatory swimming modes in early whales: hindlimb followed by tail domination. Newly described axial skeletal material from several early whale taxa permits this effort. Like Gingerich, we make use of a Principal Components Analysis to examine 22 linear postcranial measurements from 52 taxa. We have included the more primitive Eocene artiodactyl *Diacodexis* in addition to the anthracothere *Elomeryx*, and added *Pakicetus* to the original archaeocete sample that previously included *Ambulocetus*, *Rodhocetus* and *Dorudon*. Results emphasizing the long lumbus and tail vertebrae in early whales and modern dorsoventral undulators suggests that a specific stage of hindlimb dominance did not occur in early cetaceans.

Outcome: Oral presentation by Amy Maas at Evolution Of Aquatic Tetrapods 4th Triannual Convention, May 16-20, 2005, in Akron, Ohio.

Faculty Development

Eight faculty members at Hiram College participated in HHMI-supported activities in the first year of this grant, with no members added to the grant team. The short paragraphs below outline the contributions of each faculty member. At the end, some challenging aspects of faculty development for the future are described.

Brad Goodner, Associate Professor of Biology, administered the grant as program director (supported by a month of summer salary). In addition, he mentored 9 students in grant-supported independent research during the academic year and another 10 students during the summer (supported by academic year and summer stipends to students as well as research supply funds). **Cathy Wheeler**, Research Teaching Associate of Biology, also mentored many of the same students. Working with Cathy Wheeler, Brad incorporated research into the Molecular & Cellular Biology, Genetics, and Microbiology courses (supported by course research supply funds). Finally, working with Cathy Wheeler and **Willa Schrlau**, Research Teaching Associate of Biology, he ran a 2-week research summer camp for high school students and a 2-week research-in-courses workshop for high school teachers (supported by only a small amount of administrative support funds – mostly paid by participants in these first trial runs).

Kim Phillips, Associate Professor of Biology and Psychology, working with **Courtney Buzzell**, Research Teaching Associate of Biology, mentored 3 students in grant-supported independent research during the academic year and another 5 students during the summer (supported by academic year and summer stipends to students as well as research supply funds). In addition, she used one month of grant-supported summer salary to work out protocols for ELISAs and other microtiter dish-based assays to be incorporated into a new behavioral endocrinology course.

Sandy Madar, Associate Professor of Biology and Assistant Academic Dean of the College, mentored 2 students in grant-supported independent research during the academic year and another 3 students during the summer (supported by academic year and summer stipends to students as well as research supply funds). In addition, she organized and ran a one-day workshop for high school teachers on teaching evolution (supported by only a small amount of administrative support funds).

Prudy Hall, Professor of Biology and Chemistry, incorporated research into her Biochemistry I and Biochemistry II courses (supported by course research supply funds).

Colleen Fried, Professor of Chemistry, used one month of grant-supported summer salary to work out protocols for using a new microwave organic synthesis accelerator labstation in introductory and intermediate organic chemistry courses.

Curriculum, Equipment, and Laboratory Development

In grant year 1, funds were used to modify or enhance 5 courses at Hiram College involving 3 faculty members, to purchase 3 large pieces of equipment, to make a few small laboratory renovations, and to change the status of 3 staff members involved in the grant. The following sections deal with these grant-supported activities.

Course Modification/Enhancement:

Incorporation of original research into courses is one of the central themes of our HHMI grant and builds on previous success at Hiram College through the Hiram Genomics Initiative. In grant year 1, 3 faculty members (Brad Goodner, Cathy Wheeler, and Prudy Hall) incorporated research projects into a total of 7 courses (2 iterations of Molecular & Cellular Biology, 2 iterations of Genetics, Microbiology, Biochemistry I, and Biochemistry II) involving 108 students. Each within-course research project is described below.

On October 8th, 2004, Hiram College hosted a symposium entitled "Annotation of the *Chromohalobacter salexigens* Genome". The symposium brought faculty from Hiram College, Purdue University, and the U.S. Department of Energy Joint Genome Institute together along with Hiram students as part of an ongoing collaboration to sequence and annotate the genome of one of the most halotolerant members of the domain Bacteria. A total of 49 students in 3 courses at Hiram College participated in bioinformatics research during the first half of the semester and presented their results in poster sessions at the symposium. Students in the Molecular & Cellular Biology course reconstructed metabolic pathways from the *C. salexigens* genome annotation and compared those pathways with those from related bacteria. Students in the Genetics course compared *C. salexigens* and its close relatives in terms of gene order and possible regulatory circuits for metabolic pathways. Students in the Biochemistry course compared the isoelectric points for predicted protein sequences across four large cellular localization groups (cytoplasmic, plasma membrane, periplasmic, and outer membrane) to test the hypothesis that extracellular proteins of halophilic organisms have adapted to a more negative overall charge that prevents their precipitation under high salt conditions.

During the Fall 2004 and Spring 2005 semesters, 48 students in the Molecular & Cellular Biology course conducted functional genomics research on the well known soil bacterium and plant pathogen *Agrobacterium tumefaciens*. Teams of 2-3 students were assigned a gene with a predicted cellular function based on the *A. tumefaciens* C58 genome sequence. Over the course of the semester, each team amplified a portion of their assigned gene by PCR and cloned it, used their cloned sequence to disrupt the wildtype gene in *A. tumefaciens*, designed and carried out 1-2 experiments to test the putative function of their assigned gene, and presented their results. A total of 18 genes were disrupted and the mutant strains initially characterized. These mutants have been used in future courses, in independent research, and will be distributed to colleagues at other institutions.

Throughout the spring 2005 semester, 27 students in the Genetics course at Hiram College participated in the annotation phase of a genome project on three *Agrobacterium* strains that span the evolutionary breadth of the genus. Groups of 3-4 students were assigned biosynthetic pathways for strains C58 (biovar 1) and S4 (biovar 3). Each group 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. They then determined if C58 and S4 had all the necessary genes, whether any genes were

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missing at the bioinformatic 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. For “How Do Genes Change”, students used gene-based phylogenetic trees to search for C58 and/or S4 genes that did not have strong homologs in *Agrobacterium*'s closest sequenced relatives.

Three students in the Fall 2004 Genetics course worked out the protocol for transposon insertion mutagenesis of *Xenorhabdus nematophila*, a gamma-Proteobacteria symbiont of certain nematodes and pathogen of insect larvae. The students used transposon mutagenesis to conduct a screen for mutants affecting phase variation of various extracellular enzyme activities.

During the Spring 2005 Genetics course, 27 students worked to pick over over 2500 transposon insertion mutants of *Agrobacterium vitis* S4 and construct a genomic DNA set for pools of the mutants to be used in PCR-based screens for transposon hops in specific genes.

Four students in Biochemistry II focused on an assay for the gluconeogenic enzyme 1,6 biphosphatase activity. This enzyme activity must be present in both *Chromohalobacter salexigens* and *A. tumefaciens*, even though there are no known homologs in either genome. Sarah Zilka then took a 3-week research course with me in the spring of 2005. The students were able to show convincing evidence for the enzyme activity in both of these organisms and this assay can form the foundation for a mutant hunt in subsequent years.

Ten students in Microbiology continued our long-running sampling of Silver Creek, a small stream running through the J.H. Barrow Field Station run by Hiram College. The students collected samples from a range of stream strata and microhabitats, characterized the overall microbiota in terms of metabolic potential and antibiotic resistance, and purified and identified over 25 strains using culture-dependent and culture-independent methods.

Equipment Purchases:

Three major purchases were made in grant year 1 that will impact many courses and independent research projects in the future. A microwave organic synthesis accelerator labstation (Milestone Inc.) was purchased to be used by students in 1) the introductory and intermediate organic chemistry courses to speed up multi-step syntheses, and 2) independent research projects. Colleen Fried used one month of grant-supported summer salary to start working out protocols for this apparatus. A microplate reader with absorbance, luminescence, and fluorescence capabilities and a microplate washer (Dot Scientific, Inc.) was purchased for use in multiple courses (e.g., Behavioral Endocrinology, Molecular & Cellular Biology, Biochemistry I & II). Kim Phillips used one month of grant-supported summer salary to start working out protocols for this apparatus. Finally, a 10-node server cluster (PSSC Labs) was purchased for use in bioinformatics applications in several courses and independent research. One student, Adam Ewing, worked under the supervision of Brad Goodner to setup and configure the server cluster.

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Laboratory Renovations:

Minor renovations were made to 3 rooms in Colton Hall on the Hiram College campus to better serve the needs of grant-supported activities. An old chemistry lab that saw limited use was updated with some shelving on individual benches, air-conditioning, and a new refrigerator to house expanded lab sections of the Molecular & Cellular Biology, Genetics, and Microbiology courses. An adjacent small storage room was converted to house the 10-node server cluster with air conditioning put into it and the adjoining lab space to better regulate the temperature for these critical pieces of equipment.

Upgrade of Staff Positions:

The 9-month Instructor positions held by Courtney Buzzell, Willa Schrlau (formerly Ewing), and Cathy Wheeler were upgraded in title and pay using grant funds to 12-month Teaching Research Associate positions.

Precollege and other Outreach Programs

Three faculty members at Hiram College (Brad Goodner, Cathy Wheeler, and Willa Schrlau) participated in HHMI-supported precollege and other outreach activities in the first year of this grant. The short paragraphs below outline four activities in this component of the grant.

During the academic year, Brad Goodner and Cathy Wheeler continued a long-running outreach collaboration with biology teacher Diane McBeath at Cleveland Benedictine High School. Ten students in an advanced biology class received training on basic microbiology techniques and transposon mutagenesis. The students then spent the academic year, as their course syllabus allowed, working on the isolation and characterization of random transposon mutants in the nematode symbiont *Xenorhabdus nematophila*. At the end of the academic year, the students visited Hiram College for a day of genomic DNA isolation, pulsed-field gel electrophoresis, and recombinant DNA cloning.

Due to the revisions required to bring our overall grand budget into line, we had to move back the initial summer teacher workshop until grant year 2. However, we had sufficient interest to warrant funding an initial run of workshops through a pay-as-you-go approach. In one workshop, 7 high school students ranging from rising sophomores to rising seniors participated in a 2-week research opportunity. The students were housed on the Hiram College campus and participated fully in the day-to-day research activities of the Goodner research lab mentored by Brad Goodner and Cathy Wheeler. The students received training in basic microbiology techniques, recombinant DNA cloning, and bacterial genetics, then applied their learning to the construction of a genetic and physical map of the *Azotobacter vinelandii* chromosome. The students left the workshop with their advanced training, an increased awareness and appreciation for the role of research, and a poster to take to their home high school describing their project. In the second workshop, 3 teachers from local high schools participated in a 2-week research opportunity mentored by Brad Goodner, Cathy Wheeler, and Willa Schrlau. The high school teachers received training in basic microbiology techniques, recombinant DNA cloning, and bacterial genetics. A significant portion of the workshop was dedicated to brainstorming about research projects that would best fit into the courses taught at each participating high school. The teachers then worked on the protocols needed for two separate projects: (1) the isolation and characterization of *Agrobacterium* strains from plant crown galls harvested from the wild, and (2) the construction of a genetic and physical map of the *Azotobacter vinelandii* chromosome. All 3 teachers agreed at the end of the workshop to participate in grant-supported outreach collaborations with Hiram College during grant year 2.

The final outreach activities were aimed at our colleagues working in undergraduate education. Brad Goodner made two presentations, one poster and the other an invited talk, at the 2005 ASM Conference on Undergraduate Education and the 2005 ASM General Meeting (Division W Symposium on Teaching Bioinformatics), respectively. Both of these presentations focused on methods for teaching bioinformatics in a wide variety of undergraduate courses and linking bioinformatics to wet lab activities.