Summer 2010: Circadian Rhythms in a Cave Obligate Isopod

The summer of 2010, I performed molecular genetics research in collaboration with Professor Zigler, an invertebrate zoologist and member of the Biology Department Faculty here at the University of the South. We were concerned with approaching a question regarding the evolution of a cave-dwelling crustacean, *Caecidotea bicrenata*, and whether or not this species conserves a metabolic pathway characteristic of most organisms dwelling in surface environments. To answer our question quantitatively, we would need to do the following: collect *Caecidotea bicrenata* from their habitats in various caves on the Domain, round up other members of the genus *Caecidotea* for genetic comparison as a positive control, amplify desired portions of selected genes, and finally, sequence and quantify the amplified sections of their genome. I must say that during my six weeks internship, I was not able to complete half of these tasks. As I will describe later, this scope of this project extends beyond just the summer, but occupies my efforts in the semesters following the internship period.

Most people have heard of circadian rhythms in some manner, whether through the media or a scientific encounter. Circadian rhythms are commonly thought of as the internal body clock. They contribute to one's sleep cycle, alertness, tiredness, hunger, and a sum of bodily activities in the rise and fall of a single day. Interestingly enough, not only does some form of the internal body clock exist in most organisms, but also the types of molecules that govern the internal clock are fairly similar in type and structure in very diverse creatures. That humans, fungi, and even archaebacteria share a similar metabolic component indicates that the evolution of the Circadian Rhythm Pathway early on in the history of life on Earth. Whenever the evolutionary event occurred, it eventually

led to the creation of a negative feedback mechanism of a protein called CLOCK, a transcription factor that governs the expression of the downstream effecter genes it modifies. The activities of each downstream gene can be numerous depending on the organism. However, the pathway is activated through a single event: exposure to light. A different, photoreceptive protein triggers degradation of CLOCK when exposed to a characteristic wavelength of UV radiation. A cycle of building up and breaking down CLOCK creates expression waves of downstream genes, which are ultimately understood as governing the 24-hour body clock.

Dr. Zigler and I explored a possible exception to this inherent metabolic event. We ask, “If cave organisms have evolved to lack photoreceptive structures, their eyes, could they have also evolved to lose the Circadian Pathway? If present, what governs it? What sort of daily rhythm do cave-dwelling organisms follow, if one at all?” We were driven by the curiosity of affirming a common trend in the force of evolution: the tendency to strip the organism down of its unnecessary components, to eliminate and streamline it to the bare essentials. Collecting data to quantitatively determine whether or not circadian genes existed in the cave obligate crustaceans, we extracted genetic information from organisms then attempted to amplify a few regions of *Caecidotea*’s genome. We chose a handful of genes in the pathway to amplify.

My internship weighted heavily towards laboratory rather than outdoor investigations, namely performing the Polymerase Chain Reaction. The Polymerase Chain Reaction, or PCR, is a gene amplification procedure that mimics the natural process of DNA synthesis. Needless to say, I was fascinated with this process; it is a successful human attempt at reverse-engineering the manner in which all organisms create copies of their

genetic information. The caveat with PCR is that in order to “fish” out the appropriate sequence, separating in from the entire genome, one needs to begin with the perfect “fish hook” – the primer sequences. Designing a group of primer sequences was the most challenging part of my job, as it is an expensive shot in the dark. To design a primer, it is necessary in researching the availability of gene sequences in a variety of organisms, closely and distantly related to *Caecidotea bicrenata*. When this information is not available, PCR primers are less likely to be the appropriate “fish hook” and no portions of the genome will be amplified.

This summer, I performed over 280 individual Polymerase Chain Reactions. I was free to set up and undertake lab experiments at my leisure. My schedule was created independently once I knew the basics of the molecular techniques. My mornings usually began early, arriving to Spencer Labs at 7:30am. Every day at this time, I would check the previous afternoon’s PCRs for amplified sequences using Agarose Gel Electrophoresis, a procedure in which DNA fragments are separated in a gel medium according to size. After confirming the success, failure, or ambiguity of the previous reaction, I would set up another PCR and tweak the amounts of reagents to optimize the experiment. A PCR reaction takes anywhere from 2-3 hours and I was able to run 2 sets of PCRs daily. I would get out of the lab sometimes at 4pm, sometimes at 6pm depending on the circumstances.

As was expected, I ran into difficulty amplifying circadian genes due to faulty primer sequences. Retrying the same experiment demanded a great deal of patience. I always waited with anticipation upon the revealing moment, placing the agarose gel in the reader. Often failing, I found that I maintain a great deal of hope in what remains unseen.

I knew I embodied this trait, a blind faith in something of my choosing. It allowed me to fail and retry repeatedly. Though I never successfully sequenced a circadian gene in isopods, I'm optimistic in knowing that there is a way to do it – human just have not caught on yet.

The most adventurous responsibility of mine was the conquest of *Caecidotea* on the Domain: plunging through ponds, creeks, and below the earth's surface in search of tiny monsters. The most mysterious cave explored bears the gruesome name "Buckets of Blood." The chasm located a couple hundred feet off 41-A as it climbs from Cowan to Sewanee, Dr. Zigler and I crawled in to find isopods flourishing in the streambed. There were hundreds every square meter, a very dense population. This became our *Caecidotea bicrenata* headquarters. It was my favorite cave solely because of its name. To mention Bucket of Blood in casual conversation made it seem as though I just navigated an underground, haunted pit with snakes, demons, vampires, and all the rest. I found great satisfaction with the act of crawling into the earth's layers and peering on the ancient natural history embodied in the rock and soil, sustaining the life atop.

Spoiled with an abundant *Caecideotea* population in Buckets of Blood, we had to work a little harder to find isopods in other streams and caves. One finds the entrance to Walker Springs Cave well below the Cumberland Plateau north of Shakerag Hollow. It is a trap for the porous limestone above it, continuously filtering out impressive volumes of water each day. To climb into Walker Springs, I needed to submerge myself to the neck in the cave stream and crawl through a tiny passage with little air to breathe. This is the moment where you question whether it is a good idea to continue on; your body usually answers "NO!" as you become immersed in fifty degree water but your curiosity

endeavors forward. Walker Springs offered stunning cathedral walls and challenging scrambles. It is a Mecca for local cavers now and long ago, evident by the graffiti made by carbide lanterns of identifiable old Sewanee names and fraternities. I am intrigued by the history of biology and the environmental sciences here in Sewanee. Reading and hearing stories about the hydrogeology on the plateau, the first caves discovered, and the early studies of cave creatures, I get a sense of how the practice of scientific investigation unfolded in Sewanee.

Although I did not complete the entire scope of my project, amplifying and sequencing circadian genes, I enjoyed the preliminary stages very much, no matter how many times repeated. Working in the lab allowed me to feel competent that I had the skills to gather data and address questions on the microscopic scale. I have taken many biology classes with labs but no experience can compete with planning experiments and the designing the entire scope of one study. If I had pursued biological research outside of Sewanee, say with through a large university or through an NSF internship, I could not have designed my own study, chosen a species in question, generated questions, create a method, and hatched a plan of action. I learned something new daily as the opportunities for casual and scientific conversation with Dr. Zigler were phenomenal.

The primary goal of my summer was to discern if I had a passion for research, especially addressing questions of molecular biology. The past two summers, I have explored a career in medicine as my possible vocation. I am still on the pre-med track with my classes, have taken the MCAT, and applied to 13 medical schools. In previous summers, I figured out that I am called to serve humanity in some regard, in serving broken communities, feeding the famished, or providing roof to the wanderer. Over the

course of the summer, I was surprised to find that I enjoy the empty laboratory and the pursuit of knowledge driven by a semi-selfish intellect, simply a hunger for exercising the scientific method. I envision myself performing research again in the future. I would be interested in continuing the biochemistry theme of invertebrates by possibly researching the life cycles of parasites and their impact on human health in the tropics. If I did not pursue medical school immediately after college, I would apply to public health schools that emphasize this sort of fascinating pathogenic bug research. Though I fell short of supporting my hypotheses, this summer instilled in me the great hope that the scientific method is at my fingertips and a deep respect for all that is unknown and to-be-elucidated still by the human mind.