

## **UGA Libraries Undergraduate Research Award 2012**

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*Geonomic Analysis and Drug Sensitivity of Histomonas meleagridis*

### **Introduction**

To my dismay during my third year at UGA, I was not allowed to enroll in the lecture section of the biotechnology class offered every spring semester without also being enrolled in cellular biology, a class usually reserved for the final semester of senior year on pre-medical students' hectic schedules. Happily, I found that a lengthy laboratory section of biotechnology lacking the same requisites was adjunctively offered, and I jumped at the opportunity. My progress since that first hands-on introduction to methods in biotechnology in the lab has extended beyond the typical undergraduate research experience for the life science majors – I promptly and enthusiastically joined the lab of Dr. Robert Beckstead, one of the three instructing professors for BTEC3000L, and immersed myself in a new research topic with which I had no prior experience.

Immersion truly is the proper descriptor for the past 50 weeks of my involvement in the activities occurring in room 119 of the basement of the Poultry Sciences building, as I progressed from the simple (preparatory steps and reading materials and experiments to be later performed by my peers in the BTEC class) to the complex (authoring two individual scientific papers on my independent research projects on *H. meleagridis* for publication as an undergraduate student).

Dr. Beckstead initially presented me with a wealth of information – over five thousand sequencing transcripts from a cDNA library that he had recently created, neatly encapsulated in a 3MB Microsoft Word document. In formulating my own research project, he had some tentative suggestions for data mining the new sequencing information – or extracting useful details about the genome of *Histomonas meleagridis* – but I, knowing little about either bioinformatics or protozoan parasites, decided the best place to start was with a truly exhaustive review of all the available resources and literature concerning the topics at hand.

### **Bioinformatics**

Since my initial spike of interest just before beginning high school, I have developed a strong background in both the hardware and software of computer systems and networks. I work part-time as a student technology consultant for Enterprise Information Technology Systems and independently repair laptops in my leisure time to help pay for school (I love taking things apart to learn how they work, but it's much more satisfying if I may solve a problem – or two – in the process). With such a skillset in hand, I first began working on the suggested bioinformatics analyses of the DNA sequences I had been provided. I searched for similar *in silico* projects with comparable goals and software applications that might facilitate the research and data flow and was soon overwhelmed. I regretted that my programming

skills were limited, keeping me from using effectively some of the more common and well-developed open-source bioinformatics applications based on Haskell, Ruby, Perl and Python programming languages – such as the BioPerl suite. In my searching, I found these to be highly customizable, often updated, and based on a large community of front-end users, each of whom might make minor modifications in order to add to the functionality of the core application. Thanks to fluency with Boolean operators and some deft searching, I was successful in finding a handful of useful programs that did not require relatively foreign programming fluency or a large payment. Countless hours of iterative searching produced the Java-based applications Sequence Manipulation Suite 2, BLAST2GO, and Geneious, each of which I used extensively in data-mining the cDNA library. Using these programs, I was able to glean a great volume of annotative information from the provided sequencing data: codon usage statistics, putative polyadenylation signals and open reading frames, coding sequences for protein products, distinctive bacterial background in *Histomonas* culture and more (all of which is presented in my first research paper). Data workflow was unorganized until I mastered manipulation of the more common bioinformatics file formats – using a combination of these applications and some Microsoft Office macros for Excel, I was able to parse the relevant information into a simple spreadsheet that could be viewed remotely.

The search for stand-alone applications was inspired by a new resource Dr. Beckstead had shown me – NCBI's BLAST searching, or the computer-assisted generation of local alignments in DNA sequences and their analysis for homology across all species with reference DNA sequences in the NCBI database. Unfortunately, NCBI's BLAST searching is webpage-script-based and does not allow for multiple, sequential, automated alignments (within a 30 second window), making the identification of our 5,000 contiguous DNA reads a daunting, mindlessly time-consuming task. BLAST2GO, which allowed for the assembly of a large number of contigs into a structured database, completely eliminated the problem with its automated "batch" BLAST searching function, effectively enabling me to translate our inscrutable sequences overnight into a goldmine we could readily analyze the following morning. From this enormous, annotated database, we could then begin playing with the molecular biology of *Histomonas* as never before, and furthermore, we could even provide others with similar research interests with this newly synthesized resource upon submission of this data to NCBI's repository of genetic information, GenBank. This annotated data would not only launch several studies in our lab, but would also be available for the benefit of future research and studies in other parasitology labs.

### ***The metabolism of Histomonas meleagridis and zinc treatment***

Moving from the *in silico* work to the *in vitro* work, I broadly surveyed all of the available literature concerned with *Histomonas* using comprehensive searches via GIL, ILL, Pubmed, NCBI, Google Scholar, and Web of Knowledge. At this time, I was receiving credit for biochemistry major-related research for my work and hence decided to focus on studying the metabolism of *Histomonas*, which is centered about the hydrogenosome, or the anaerobic equivalent of mitochondria. Such an organelle, both vital to the parasite's metabolism and yet distinct from the host's metabolism, presented a large target for therapeutics in prevention and treatment of infection.

Knowing very little about alternate metabolic schemes in obligate anaerobes, and based on the relative dearth of information on *Histomonas* and its metabolism, I began a literary review of closely-related organisms with similar metabolic plans. *Trichomonas vaginalis* and *Tritrichomonas foetus* were similar parasites of the same phylogenetic order with a greater number of publications that proved invaluable to current research on treatment and prevention of histomoniasis. Following a landmark paper published in the 1970s by Lindmark and Muller, I delved into the footnotes and cited references of all papers mentioning protozoan hydrogenosomes to develop the necessary (and more contemporary) background knowledge for comprehensive analysis of the most promising avenues of research on the organism of interest. Most of the general biochemistry of hydrogenosomes was elucidated by the 80s, but some details remain unclear for particular, non-model organisms. While *Histomonas* has not had its entire genome sequenced or assembled to date, *Trichomonas vaginalis* has had a complete genome available for several years now, and its entire proteome has been outlined. Of particular interest to me was the hydrogenosomal proteome of *T. vaginalis*: I began cross referencing our database of BLAST results with those proteins known to be specific to the *Trichomonas* hydrogenosome in an attempt to identify homologous *Histomonas* hydrogenosomal proteins, with significant success.

With some academic background in the biochemistry of hydrogenosomes established, I set out to find a way to disable this important organelle. The current standard of treatment for *Histomonas* infection is metronidazole, a drug that acts directly upon hydrogenosomal proteins and is banned in the USA and Europe for use in animals bound for human consumption. In my literature review, I found a single paper that took this biochemical approach and broadened it to probe for compounds lacking the carcinogenic properties of metronidazole that might work similarly within the parasite. Marlene Benchimol authored a series of papers, one titled "The Hydrogenosome as a Drug Target," that pointed my research in the right direction. The application of zinc at variable concentrations had a disabling effect on *T. foetus* – an effect verified to be localized to the hydrogenosome via electron microscopy. Unfortunately, less than a handful of publications mention zinc and hydrogenosomes. I was shocked to see such a successful lead with little further research, and attempted to translate the finding to *Histomonas*.

After countless drug sensitivity assays, we have finally determined that the previous finding in *T. foetus* applies similarly to *Histomonas* and discovered the boundaries of hydrogenosomal sensitivity to the otherwise innocuous zinc sulfate. Two separate publications based upon this long year of research are forthcoming, and I was delighted to browse parasitology and other relevant journals for their impact factors for submission of these papers as an undergraduate student. Translational studies are now in progress, attempting to find an effective delivery method to inoculate infected turkeys *in vivo*.

Without a solid foundation of literary research methods, clearly none of this progress would have been possible. To date, it surprises me that the 1993 Benchimol paper seems to be greatly overlooked in the field of parasitology, and that no one else has compounded such findings. Parasitologists and biochemists and bioinformaticians together can further unravel the mysteries of *Histomonas*. Using an interdisciplinary approach to research, we will one day elucidate the detailed workings of its – and other organisms' – entire genomes.

## A new approach to histomoniasis: data mining and targeted drug sensitivity

*Histomonas meleagridis*, the causative agent of blackhead disease in gallinaceous birds, is an anaerobic parasite that lacks mitochondria. Few treatment options exist for *Histomonas* infection, the most effective of which is banned in the USA and Europe for application in livestock bound for human consumption. Previous studies on closely related organisms have revealed certain details of their anaerobic, hydrogenosomal-centered metabolism, providing a clear target for drug treatment that is distinct from the host's metabolism. Using a multi-disciplinary approach, we have generated a cDNA library of the *H. meleagridis* genome and an annotated contiguous DNA sequence database to study virulence factors and specific metabolic components. Additionally, we examined the potential and efficacy of novel methods of controlling infection by targeting the hydrogenosome. In this study, we demonstrate through a series of sensitivity assays that application of variable concentrations of zinc in solution significantly inhibits *in vitro* growth by acting on the hydrogenosome. Furthermore, we initially evaluated potential methods of zinc delivery *in vivo*.

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