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***Tetrahymena* in the Classroom**

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Abstract

Tetrahymena has been a useful model in basic research in part due to the fact it is easy to grow in culture and exhibits a range of complex processes, all within a single cell. For these same reasons *Tetrahymena* has shown enormous potential as a teaching tool for fundamental principals of biology at multiple science education levels that can be integrated into K-12 classrooms and undergraduate and graduate college laboratory courses. These *Tetrahymena*-based teaching modules are inquiry-based experiences that are also effective at teaching scientific concepts, retaining students in science, and exciting students about the scientific process. Two learning communities have been developed that utilize *Tetrahymena*-based teaching modules. Advancing Secondary Science Education with *Tetrahymena* (ASSET) and the Ciliate Genomics Consortium (CGC) have developed modules for K-12 students and college level curriculums, respectively. These modules range from addressing topics in ecology, taxonomy, and environmental toxicity to more advanced concepts in biochemistry, proteomics, bioinformatics, cell biology and molecular biology. An overview of the current modules and their learning outcomes are discussed, as are assessment, dissemination, and sustainability strategies for K-12 and college level curriculum.

I. Introduction/Rationale

The last decade has seen the development of *Tetrahymena thermophila* as a remarkable tool for both teaching and research. *Tetrahymena* combines the simplicity of an easily manipulated single cell system with a structural and functional complexity comparable to that of higher metazoans. It's genetics, development, cell physiology, biochemistry, and ultrastructure are well characterized (for overviews of the system, see Elliott, 1973; Hill, 1972; Nanney, 1980; Gall, 1986), and an array of biochemical, physiological, genetic, and molecular techniques have been developed to facilitate use of the *Tetrahymena* system in basic research (Asai and Forney, 2000; chapters in this volume). As a result, *Tetrahymena* has become a well-published model for research in fundamental areas of cell biology, including telomere assembly, histone modification, programmed genome rearrangement, membrane trafficking, endocytosis, exocytosis, tubulin structure, cilia (cytoskeletal) dynamics, among others (e.g. see reviews by Turkewitz *et al.*, 2002; Turkewitz, 2004; Mochizuki, 2010; Chalker and Yao, 2010). For many of the same reasons that *Tetrahymena* has proven useful in basic research, it has enormous potential as a teaching tool. Its complex behaviors are easily manipulated in small volume cultures, and provide a convenient window into many of the fundamental principals of biology. Practical aspects of working with *Tetrahymena* make it ideal for the classroom. Most importantly, *Tetrahymena*'s free-living, non-pathogenic lifestyle makes it completely safe to work with. In addition,

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Tetrahymena exhibits a wide range of biological characteristics and activities that are suitable for investigation of fundamental biological concepts at multiple educational levels, from K – 12 classrooms to undergraduate college courses. Integrating the fruits of research with *Tetrahymena* into the science curriculum at the K – 12 and college levels provides students with access to new hands-on approaches to important scientific ideas, and benefits researchers by increasing public awareness of the relevance and importance of basic research.

Efforts to integrate *Tetrahymena* into the biology curriculum at the high school and college level have been greatly aided by several key tools, starting with organization of the genome sequence into a searchable Wiki that is easily accessed and used by students at all levels, even those just learning to navigate genome databases (<http://www.ciliate.org>). Gene expression data is also available in an easy-to-use format at the Tetrahymena Functional Genomics Database (<http://tfgd.ihb.ac.cn/>). Molecular genetic tools facilitating the straightforward engineering of epitope tagged proteins for localization and protein complex analyses have recently been developed for college level courses. In addition, *Tetrahymena*-based teaching modules incorporating bioinformatics are being developed for high school use. The development of genomic and proteomic based teaching tools is greatly facilitated by access to the user-friendly database interfaces provided by the *Tetrahymena* research community. In addition to bioinformatics resources, the use of *Tetrahymena* as a teaching tool is enhanced by the availability of a variety of strains exhibiting diverse genotypes and phenotypes (see Ch. 8 in this volume, and <http://tetrahymena.vet.cornell.edu/>) and by the ongoing development of new *Tetrahymena* research techniques that can be modified for classroom use.

At the college level, many teaching modules using *Tetrahymena* have been independently developed by faculty at a variety of institutions. A workshop called "Ciliates in the Classroom", held as part of the semi-annual Ciliate Molecular Biology research conference, was initiated over 15 years ago as a place where college teaching faculty share their ideas, protocols, and resources, and collaborate on larger teaching initiatives. In parallel, national recommendations for effective biology teaching reform evolved from federal and private organizations, including the American Association for the Advancement of Science (AAAS), National Research Council (NRC), Howard Hughes Medical Institute (HHMI), Project Kaleidoscope (PKAL), and others (Brewer and Smith, 2011; Fairweather, 2008; NRC, 2003, 2009, 2011). These tout inquiry-based experiences for students as effective ways of both teaching scientific concepts and retaining students in science. Members of the ciliate community saw an opportunity to address these recommendations by developing ciliate-based research activities into scalable modules for integration into college classrooms at multiple levels. Initiated through the workshop discussions, a fairly extensive set of modules now exist, which engage students in the excitement of original research on a variety of biological questions, while simultaneously teaching central concepts in cell and molecular biology, biochemistry, ecology, and taxonomy.

Recommendations for transforming biology teaching to retain students in science included the building of learning communities, which can help inspire and support students with a variety of backgrounds in science (NRC, 2009; AAAS, 2011). In particular, learning communities are supportive of under-represented students such as women and racial minorities (Treisman, 1992; Jones *et al.*, 2010). *Tetrahymena* biologists have recently capitalized on this idea by involving undergraduate students directly in the functional annotation of *Tetrahymena* genes through the research modules described below. Faculty and students engaging these modules are part of the Ciliate Genomics Consortium (CGC), which was initiated in 2006 with funding from NSF. Participating students can immediately disseminate and share their results with the broader ciliate community through a database for

unpublished results (<http://tet.jsd.claremont.edu/>) that is linked to the *Tetrahymena* Genome Database. Through this mechanism, students are immediately connected with and part of the larger research community. Assessment has shown that this experience has validated their ability to make real scientific contributions and has empowered them to immediately pursue further research experiences (Wiley, unpublished).

Tetrahymena has also been featured in teaching exercises that can be used in middle and high school classrooms (e.g. Bozzone, 2000; http://www.ascb.org/newsfiles/exercises_cell_bio.pdf), and has been utilized in high school science fairs (e.g. http://www.all-science-fair-projects.com/science_fair_projects_encyclopedia/Tetrahymena_thermophila; Intel 2011 Science Fair, <http://www.societyforscience.org/document.doc?id=295>) and other independent student projects that encourage more in depth student involvement with scientific concepts, and stimulate enthusiasm and commitment beyond the standard science classroom format. Currently, K – 12 educational use of *Tetrahymena* is being expanded under the auspices of an NIH SEPA funded program (ASSET: Advancing Secondary Science Education with Tetrahymena; <http://tetrahymenaasset.vet.cornell.edu/>), specifically designed to develop *Tetrahymena* based laboratory modules targeted to middle and high school students.

In this chapter we outline the classroom research modules that are available at multiple levels, and their learning outcomes that align with the most recent recommendations for transforming biology education. Additionally, we discuss assessment outcomes and future plans to improve sustainability and strengthen interactions between students in the research community and faculty at a variety of institutions.

II. Materials and Methods

II.A. Tetrahymena in the College curriculum

The following classroom research modules have common learning outcomes:

1. Develop facility with designing experiments; improve understanding of controls;
2. Improve data analysis, interpretation, and presentation skills;
3. Learn effective scientific communication and general collaboration (teamwork) skills;
4. Improve scientific writing skills;
5. Deal with ambiguity through engaging original questions; and
6. Aid in the structural and functional annotation of the *Tetrahymena* genome

All of the following modules and instructor guides are available on the Ciliate Genomics Consortium (CGC) website (<http://www.tet.jsd.claremont.edu>)

II.A.1. Current modules

Bioinformatics modules (requires 3–6 hours of class time): These modules guide students through identification of a gene(s) of interest that belongs to a particular gene family, or that may be a homolog of a specific gene in another organism. Students analyze gene sequence for features such as splice sites, translation start and stop codons, and analyze alignments of Expressed Sequence Tags (EST) to evaluate the gene structure predictions. Students predict protein features through a variety of free online programs (Figure 1A). Students can compare protein sequences and construct phylogenetic trees (Figure 1B). The bioinformatics modules may be used independently or as precursors to other modules.

Students learn to retrieve gene information from various databases, the difference between genomic and coding sequences, gene structure (start and stop codons, introns and exons), the concept of expressed sequence tags, the use of gene translation tools, organism-specific codon usage, BLAST searching, sequence alignments, concepts relating to functional domains, and evolutionary sequence conservation.

Gene Structure Determination (5 × 4 hour laboratory periods): The coding sequences of all putative *Tetrahymena* genes have been computationally predicted. These predictions now require experimental testing. In this module, students work in teams to identify introns, exons, and the 5' and 3' termini of gene transcripts and coding sequences by PCR.

Students learn standard molecular techniques (primer design, PCR, agarose gel electrophoresis, genomic DNA isolation, RNA isolation, cDNA synthesis, nucleic acid quantification), the difference between genomic and coding sequences, introns vs. exons, basic gene structure, the concept of 3' and 5' untranslated regions, effective data presentation, and the use of gene graphics software.

Gene Expression Analysis (5 × 4-hour laboratory periods): In this module students assess the relative amount of expression of a gene of interest throughout different stages in the *Tetrahymena* life cycle. This takes advantage of processes and physiological changes that are synchronized through conjugation. Gene expression is evaluated through assessing the relative production of gene transcripts at different time points in the life cycle by reverse transcriptase PCR and agarose gel electrophoresis (Figure 2A). Alternatively, expression can be measured using quantitative Real-Time PCR to graph the relative levels of expression under various treatment conditions (Figure 2B–D). Results can be compared to microarray-based expression profiles published on Tetrahymena Genome Expression Database (TGED; <http://tged.ihb.ac.cn/>; Miao *et al.*, 2009)

Students learn standard molecular techniques (genomic DNA isolation, RNA isolation, cDNA synthesis, PCR, primer design, agarose gel electrophoresis, nucleic acid quantification), cytological analysis by fluorescence microscopy, effective data presentation, and use of graphics software.

Protein Localization (6 × 4-hour laboratory periods): In this module students use PCR to clone a gene to fuse with a fluorescent protein (GFP, RFP, YFP, or CFP). This can be done with conventional restriction enzyme digestion and subsequent ligation or through the use of Gateway technology and LR recombinase methods (adapted from Invitrogen technology). The tagged gene (under the cadmium-inducible *MTT1* promoter) is then transformed into *Tetrahymena* either through electroporation or use of biolistic particle bombardment procedures. Positive clones obtained through drug selection are induced with CdCl₂ to express the tagged protein, and fluorescence microscopy is used to determine localization of the protein in the cells (Figure 3). Results for protein localization to various organelles and structures in *Tetrahymena* improves the functional annotation for that protein.

Students learn standard molecular techniques (PCR, agarose gel electrophoresis, restriction enzyme digestion, cloning), *Tetrahymena* transformation techniques, fluorescence microscopy, data collection and analysis, cellular organelles and structures, preparation of figures, and data presentation.

Protein Interaction through Immunoprecipitation and Mass Spectrometry (6 × 4-hour laboratory periods): In this module students use PCR to clone a gene with an epitope tag (2HA, FLAG, HIS₆, etc.) fused to it. This can be done with conventional restriction enzyme digestion and subsequent ligation or through the use of Gateway technology and LR

recombinase methods (adapted from Invitrogen technology). The tagged gene constructs (under control of the cadmium inducible MTT1 promoter) are then transformed into *Tetrahymena* either through electroporation or use of biolistic particle bombardment procedures. After drug selection of positive clones they are induced with CdCl₂, protein extracts isolated, and immunoblot analysis performed to determine expression of the tagged protein. Extracts are then used in an immunoprecipitation assay to isolate proteins that interact with the tagged protein via mass spectrometry (Figure 4). Results can help annotate the protein complexes and their possible functions in the cell.

Students learn standard molecular techniques (PCR, agarose gel electrophoresis, restriction enzyme digestion, cloning), *Tetrahymena* transformation techniques, protein isolation techniques, SDS-PAGE, western blot analysis, Coomassie staining, data collection and analysis, preparation of figures, and presentation of data.

II.A.2. Assessment

Assessment of college modules: The research modules have been integrated into the laboratory component of various courses at different levels, including: upper division molecular biology courses with laboratory, a research-based laboratory course for students in their second year, and a first-year honors life sciences course. Assessment data has been collected for over four years using attitudinal surveys, confidence surveys, written reports and oral presentations, empirical observations, tracking student placement into research positions, Student Assessment of Learning Gains (SALG; Seymour *et al.*, 2000) and published metrics for evaluation of student research experiences such as the Survey of Undergraduate Research Experiences (SURE; Lopatto, 2008).

These various assessments show significant gains in the following areas: (1) increased confidence with each step of the scientific process: generating hypotheses, experimental design and execution, data analysis, data presentation, scientific writing, oral presentation of results, and keeping scientific records; (2) enhanced student effort, investment in the project, and sense of making valuable scientific contributions; (3) increased interest in pursuing further research experiences and considering research science as a career option; (4) a sense of belonging to a larger research community and peer cohort in science.

II. B. *Tetrahymena* in K-12 curriculum

A number of teaching modules, primarily aimed at middle and high school students, are being developed by the NIH-funded ASSET program at Cornell University. Each module is designed to stimulate active, inquiry-driven learning of key biological concepts using living *Tetrahymena*, a eukaryotic organism that encompasses many of the biological attributes of metazoans but does not engender any of the conflicting emotions and reactions often invoked by the use of multicellular organisms in the classroom. Since it is critical that each module be effective in addressing relevant core concepts and be presented in a format that is both teacher and student friendly, each module is developed with input from active middle and high school teachers, tested multiple times in pilot classrooms, and evaluated at each step by an independent external evaluator. The ASSET *Tetrahymena*-based teaching modules incorporate many of the ideas expressed as part of the new framework for K-12 science education recently put forth by the National Research Council's Committee on Conceptual Framework for the New K-12 Science Education Standards (National Research Council 2011). Each of the three dimensions broadly outlined in the above plan are addressed. First, the modules allow students to actively engage in the practice of science, from asking questions to carrying out investigations to analyzing and interpreting data. All utilize a hands-on approach designed to help stimulate student curiosity, interest, and motivation. Second, the modules help to identify concepts that are fundamental to

developing a coherent, broad-based view of science by directly examining basic biological interactions like cause and effect relationships, structure and function, and evolutionary change. Third, the modules directly address many core ideas that are fundamental to an understanding of the life sciences, ranging from structures and process in organisms to ecology, heredity, and evolution.

Because of the ease and flexibility with which *Tetrahymena* can be utilized in the development of educational tools, *Tetrahymena*-based modules can be used to address a wide range of educational goals, addressing both scientific practice and biological content. For example, the ASSET modules address scientific practice and the scientific method by fostering student observation and formulation of questions in a meaningful and testable way, by helping students understand experimental design and the importance of variables and accurate and meaningful measurements, and by encouraging students to critically analyze data and compare experimental outcomes. The biological content of the ASSET modules is wide ranging, addressing issues from evolution to predator-prey interactions to bioinformatics to ecology and toxicology. Examples of the ASSET modules currently available or under development are briefly described below. As testing and evaluation are completed, each module is made available on the ASSET website (<http://tetrahymenaasset.vet.cornell.edu/>). These modules are presented to encourage use among educators at all levels, and to serve as models for future development of new, innovative teaching modules utilizing *Tetrahymena*'s unique biological attributes.

II. B.1. Current Modules

Micro-Evolution: The micro-evolution lab provides a rare opportunity for students to address evolution and natural selection with a hands-on experiment that can be completed in less than two weeks. The experiment utilizes *Pseudomonas fluorescens*, a common, nonpathogenic saprophyte that colonizes soil, water and plant surface environments and *Tetrahymena thermophila* to demonstrate diversifying selection in response to predator:prey interaction. The basic lab can be expanded to compare resource competition and predation as driving forces behind adaptive radiation. Students observe predator-driven real time evolution in a micro-environment in about a week. In the presence of a *Tetrahymena* predator, clear phenotypic changes in bacterial growth pattern and niche formation are observed in liquid culture, and related changes in bacterial colony formation on agar plates are easily distinguished. Colony formation is dependent on what niche bacteria occupy in the liquid culture, e.g. surface biofilm compared to bottom dwellers.

Students learn mechanisms of evolution; population dynamics; diversity of organisms; mutation; differences between prokaryotic and eukaryotic cells; interdependence in nature; and continuity and change.

Phagocytosis: The phagocytosis lab uses *Tetrahymena* to investigate the processes of ingestion, phagocytosis, and vacuole formation in cells, and the effects of various factors on these physiological processes. Students monitor vacuole development during feeding using digital cameras to record the data, learning microscopy and data collection and analysis as an integral part of the exercise. Students can look at feeding preferences, including live vs. dead food, big vs. small food, organic vs. inorganic material. The lab can be combined with a consideration of mutational effects by incorporating the use of mutants unable to eat for various reasons, e.g. mutants that fail to form a mouth at certain temperatures, or mutants with swimming defects that are unable to efficiently filter food from the water. The lab can also be used to address other issues like pollution and toxicology, and can be combined with labs addressing the effects of cigarette smoke and alcohol.

Students learn cellular energetics; chemistry of life; energy transfer; structural similarity between single cell and multicellular organisms; and relationship of structure to function.

Cannibalism and Interspecific Predation: The cannibalism and interspecific predation lab utilizes both *T. thermophila* and *T. vorax*. Morphogenetic change is addressed by observing the *T. vorax* shift from microstome to macrostome form. As part of the lab, stomatin, the transformation inducing substance produced by *T. thermophila* is isolated using simple techniques that can easily be carried out in high school classrooms. The change in morphology is obvious even under relatively low magnification. The lab can be used to address chemical induction of morphological changes, evolutionary differences among different species within the same genus, and, since the macrostome forms consume their own species as well as (preferably) other *Tetrahymena* species, inter- and intra-specific predation. The use of inexpensive digital cameras to record morphological changes and predation events adds enormously to student interest and enthusiasm for this exercise.

Students learn about diversity of *Tetrahymena*; relationship of structure to function; mechanisms of evolution; population dynamics; growth and survival of organisms; diversity and adaptation of organisms; inter-organismal relationships; and species variation.

Mutation: The mutation lab utilizes *Tetrahymena* strains carrying temperature sensitive mutations in genes involved in cell division. At permissive temperatures, the cells are normal, but when shifted to the restrictive temperature, they exhibit easily observed and characterized changes in morphology. This is a clear and simple demonstration of mutations, how temperature sensitive mutations work, and the effects of permissive and restrictive temperature effects in a safe, simple system. The module can be expanded to look at the long and short-term effects of exposure to restrictive temperatures and recovery parameters, and can be combined with other modules to stimulate student development of independent inquiry projects. For more advanced classes, these mutants can also be used as a basis for genetic investigation of gene dominance and gene complementation.

Students learn concepts related to mutation; gene regulation; complementation, and dominance.

Mating: The mating lab addresses issues associated with sex and reproduction from an evolutionary perspective, using *Tetrahymena* as a model system for examining sexual and asexual reproduction. The lab takes advantage of the fact that, under appropriate conditions, *Tetrahymena* can propagate either vegetatively or sexually, addressing the relative impact of genetics, growth rate, and population size on reproductive advantage. Students design an experiment to identify the mating type of unknown clones of *Tetrahymena* by testing with clones of known mating type, based on the lack of self-mating within clones of the same mating type. The effects of various environments conditions on mating behavior can also be examined, and the module can be expanded to include a discussion of chemotaxis and the role of surface proteins in cell-to-cell communication.

Students learn concepts related to population dynamics; gene regulation; science as a process; reproduction; and heredity.

Pattern Formation: The pattern formation lab deals with spontaneous pattern formation in standing cultures of *Tetrahymena*. The honeycomb patterns formed in standing cultures of *Tetrahymena* have been linked to convection, chemotaxis, and cell-cell interaction, making this module not only useful as an independent biology lab but also potentially interesting as a cross platform vehicle for linking biology, math, and physics. Since various conditions affect spontaneous pattern formation and reaggregation, including media, cell density and

environmental conditions, the lab lends itself to independent student inquiry and experimental design.

Students learn science as a process; cell–cell interactions; and cell response to environmental stimuli.

Toxicology: The toxicology lab examines the response of *Tetrahymena* cells to a variety of basic substances, looking at both lethal toxicity (LD₅₀) and chronic sub-lethal toxicity. Students can use different concentrations of common substances suggested in the module (for example, shampoo, detergents, toothpaste, Windex) to determine lethal and sub-lethal dosage, or, under teacher supervision, design their own experiment to look at common substances of interest to them. The lab offers an opportunity for students at all levels to begin to address experimental design, the effect of experimental variables, and potential long term environmental impact of even commonly used items.

Students learn about environmental impact on growth and survival; ecosystems and relationships between organisms.

Effects of Cigarette Smoke: The cigarette lab is an offshoot of the Toxicology lab that looks directly at the effects of cigarette smoke on overall cell viability, motility, and behavior, and relates the observed behaviors to ciliary activity. Cigarette smoke is bubbled into alcohol or water, and the resultant extract is added to cell cultures and the effects observed both directly and using digital image capture. Methods are presented to allow students to analyze digital images of treated and control cells by tracking cell swimming patterns using freely available software (ImageJ, <http://rsbweb.nih.gov/ij/>). A comparison between *Tetrahymena* cilia and cilia present in human lungs is presented. The simplicity of the lab design allows students considerable freedom to design experiments, and ask and answer their own questions.

Students learn concepts related to motility; cell response to environmental stimuli; and science as a process.

Effects of Alcohol: The Alcohol Lab is another offshoot of the Toxicology lab that looks directly at the effects of alcohol (beer) on overall cell viability, motility, and behavior. The effects of various concentrations of beer and the non-alcoholic equivalent on *Tetrahymena* viability and behavior are analyzed using digital image capture and cell tracking software. Students are encouraged to discuss the biology behind the observed behaviors and to design their own experiments using the cells and methodologies provided.

Students learn concepts related to motility; environmental impact on growth and survival; cell response to environmental stimuli; and science as a process.

Chemosensory Response: The chemosensory response lab addresses cell response to a variety of substances, from avoidance/attraction response to modification of ciliary beat, and presents optional sections to allow teachers to tailor module use to specific classroom levels. Middle school students examine the response of the cells to simple substances like herbs, spices, or citrus peel, some of which are provided with the kit, or they can design their own experiments to test the avoidance/attraction response to a variety of substances of interest to them. High school students can examine more sophisticated chemical responses, for example to GTP. This lab has been adapted for use in classes ranging from 4th/5th grade through AP biology.

Students learn cell responses to environmental stimuli; and interorganismal relationships.

Osmolarity: The osmolarity lab provides a clear, easy technique for identifying and quantifying cell response to changes in osmotic conditions. Contractile vacuoles in *Tetrahymena* are large and visible even with the fairly low quality microscopes generally available in high school biology labs, and changes are readily captured for detailed analysis using the single frame, time lapse, and movie options available with low cost digital cameras. *Tetrahymena* contractile vacuoles gather and expel water in periodic fashion, with the rhythm and rate of contraction dependent on environmental factors. The lab addresses membrane permeability and osmoregulation in a free-swimming cell under a variety of conditions. The exercise can be varied according to class level, from simply observing changes to collecting and graphing data on rates of contraction under different conditions to student-designed experiments altering the cell environment in specific ways and recording and analyzing the results.

Students learn concepts related to water and chemistry of life; physiological regulation; relationship of structure to function; homeostasis; feedback mechanisms; and structural similarity between single cell and multicellular organisms.

Field Research: The field research module combines field collection, cell culture, molecular biology (in the form of DNA isolation, PCR, DNA sequencing), and bioinformatics within one extended lab. It involves field collection of local *Tetrahymena* species, isolation of DNA from wild caught cells or unknown *Tetrahymena* strains (isolated from known locations) provided by the *Tetrahymena* Stock Center, PCR amplification of fragments for sequencing and bioinformatic analysis, bar coding, and examination of genetic diversity. Students have an opportunity to publish their results on the ASSET website, where information regarding all the *Tetrahymena* species identified around the country will be made available. Sequence data will permit them to identify the *Tetrahymena* species collected and place it on a phylogenetic tree. Ultimately, the information collected by students performing this module will be fed into a larger database that will provide an overview of genetic variation among natural populations of *Tetrahymena thermophila*. Students will be able to track this data as it is collected from remote sites. The lab gives students an opportunity to be part of an ongoing research project and strengthens their understanding of the scientific process.

Students learn science as a process; evolution; continuity and change; interdependence in nature; molecular genetics; diversity of organisms; and species variation.

Microscopic Life Around Us: The microscopic life around us module is a field laboratory that allows students to experience the abundant microbial life in nearby aqueous natural habitats. Students are introduced to the diversity of organisms that exist in something as seemingly simple as a drop of water, using basic keys to identify some of the more common inhabitants. Students can also collect non-aqueous material like hay or twigs, and examine flora and fauna that emerges when the objects are placed in water or various media. A more sophisticated version of the lab involves comparing the abundance and variety of life forms from various water sources, and relating differences to possible environmental factors. For example, samples from ponds treated to eliminate algae or temporary run-off ponds from agricultural sites might be compared to relatively pristine ponds or lakes, or the variation in standing versus rapidly running water examined. Students are encouraged to be creative in examining the world around them. The lab is designed to foster student interest and involvement in scientific inquiry.

Students learn experience the diversity of life; differences between prokaryotic and eukaryotic cells; interdependence in nature; ecosystems and relationships between organisms; and species variation.

Growth and Population Dynamics: The growth and population dynamics lab is purposely multifaceted. It can be used to simply address questions of cell growth, including lag, log, and stationary phases in microbial cultures, but can easily be expanded to encompass questions of population density and sustainability relative to environmental resources. The lab can be used to look at issues including population growth in response to food abundance and shortage and the effects of environmental change (temperature, salinity, water quality, introduction of competitors, etc) on population growth and maintenance. The lab exploits *Tetrahymena*'s natural lag, log, stationary, crash growth cycle, allowing students to create conditions that examine specific effects on each phase of the cycle, e.g. by input of additional nutrients, removal of nutrients, environmental changes, and changes in population density. Students are encouraged to design their own experiments asking fundamental questions about growth, population, and sustainability.

Students learn about population dynamics; science as a process; and growth and survival of organisms.

Cilia: Growth and Regeneration: The cilia growth and regeneration lab offers a unique opportunity to manipulate an important cell organelle without killing the cell. Ciliary loss can be non-lethally induced in *Tetrahymena* by several methods, and cells are capable of regenerating their cilia in a few hours. However, in the mean time they are immobile, subject to increased predation and unable to feed. The lab provides an experimental approach to examining organelle growth by chemically inducing cells to shed their cilia, and monitoring regeneration by the resumption of swimming behavior monitored by time-lapse photography or direct observation. The effects of external conditions on regeneration are easily monitored as well.

Students learn concepts related to motility; regeneration; and cell response to environmental stimuli.

Exocytosis and Secretion: The exocytosis and secretion lab deals with the induction of stimulus-dependent secretion, a topic well studied in *Tetrahymena*. This lab can also be linked to evolution of defense mechanisms in lower eukaryotes. Students induce secretion of a gelatinous capsule around the whole cell (visually similar to the jelly capsule surrounding frog eggs) in response to Alcian Blue. Cell reaction is captured by microscope mounted digital cameras using real time and time lapse exposures. Survival of the cells and ability to secrete a second capsule in response to stimulation are explored. Classroom modifications include examining the role various stimulants and calcium concentration in the secretory process. Students are encouraged to formulate their own questions and design experiments to answer them, e.g. what effect does media or temperature or cell density have on secretory behavior.

Students learn about subcellular organization; defense mechanisms; relationship of structure to function; science as a process; interorganismal relationships; and survival mechanisms.

II.B.2. Assessment

Assessment of K-12 modules: Evaluation plays a critical part in the assessment of any educational tool. For example, a full evaluation plan, compiled by an external evaluator to guide project development and assessment, is an ongoing part of the ASSET program, and is presented as a model for evaluation of future *Tetrahymena* based educational materials. The results obtained so far serve as a significant indication of the overall usefulness of *Tetrahymena* as a teaching tool. Each ASSET module undergoes both formative and summative evaluations. Formative evaluation includes working directly with those involved in developing and testing the modules to insure that the exercises are teacher and student

friendly, self-explanatory, scientifically accurate, and pedagogically sound. Testing and evaluation is carried out both in the classroom and in teacher workshops in an iterative process, with multiple rounds of revision. Teachers provide feedback to satisfy the first three criteria, and scientific and pedagogical advisors provide feedback to satisfy the final two criteria. Summative evaluation is used to determine overall impact. Teacher outcomes are determined by successful implementation of curriculum and modules in the classroom, an increase in content knowledge, an increase in the comfort level of teaching science, and an increase in use of hands-on science laboratory modules. Student educational outcomes are based on an assessment of increase in content knowledge, increase in science process skill development, and increase in understanding of scientific research. Direct classroom observation by ASSET staff and external evaluators (based on Horizon Research Observation protocol: http://www.horizon-research.com/instruments/hri_instrument.php?inst_id=14), pre- and post-testing of students in classrooms using the modules, and pre- and post-testing of teachers taking part in ASSET workshops, are all used in evaluating the program. Thus far, use of the modules has uniformly been associated with positive gains in teacher and student outcomes, supporting the use of *Tetrahymena* in the development of teacher and student-friendly laboratory exercises. Additionally, all evaluations and comments from teachers using the modules indicate that *Tetrahymena* provides a unique teaching tool that can be used in the classroom to excite students about the scientific enterprise and increase their interest in science overall.

III. Discussion

The education initiatives discussed in this chapter are inherently sustainable in their design. With an estimated ~25,000 genes in the *Tetrahymena* genome, investigating the function of each through the modules developed for college level curriculum will provide opportunity for many years of novel contributions from students. In addition, as gene knockout cell lines are created by the community, they may be tested for phenotypes using the middle- and high school level modules for novel investigations for years into the future. To enhance dissemination of student results, the Ciliate Genomics Consortium is doubling efforts to produce a user-friendly Wiki database for student-generated results. Plans to link this database with the Tetrahymena Genome Database Wiki are in progress. The CGC aims to regularly offer workshops to train faculty to use the research modules in their classrooms. The consortium is also seeking funding to support module implementation in a variety of classrooms, as they cost more than typical laboratory exercises.

As new molecular tools are developed for use with *Tetrahymena*, additional teaching modules will be developed in parallel. College level modules will be published on the Ciliate Genomics Consortium for Education website (<http://tet.jsd.claremont.edu/>), and K-12 modules will be published on the ASSET website (<http://tetrahymenaasset.vet.cornell.edu/>). Some of the modules currently in development include chromatin immunoprecipitation (ChIP), differential centrifugation of organelles, and transcription regulation through a luciferase assay.

Tetrahymena has the potential to become an intrinsic part of K-12 education, but to fulfill that promise, the use of *Tetrahymena* based teaching modules must be expanded, and a sustainable model for the continued use of these and future teaching modules must be developed. One approach that both increases the use of *Tetrahymena* in K-12 classrooms and promotes sustainability is the development of a local expert teacher program such as the one currently being developed by ASSET. Under this program, regionally dispersed expert teachers drawn from various K-12 outreach programs act as focal points for the local use of the ASSET *Tetrahymena* based teaching modules. The teachers attend an NIH SEPA funded intensive 3-day workshop at Cornell University that familiarizes them with *Tetrahymena*

biology and the standard methods needed to use *Tetrahymena* in the classroom. The workshop also provides intensive hands-on experience using the teaching modules, and suggestions for optimal methods for sharing ASSET modules with other teachers. Participants in the expert teacher program commit to providing a minimum of two training sessions on the use of *Tetrahymena* in the classroom for other teachers in their district. This approach creates a core group of knowledgeable teachers willing to share their *Tetrahymena* expertise with other teachers, expands interest in the use of *Tetrahymena* in the classroom among active teachers in disperse geographical locations, and informs other educational outreach programs about possible collaborative interactions involving *Tetrahymena*. An interactive website dedicated to K-12 educational use of *Tetrahymena* provides discussion groups and a blog for further support of participating teachers. Webinars provide an inexpensive mechanism for providing addition information on new or revised modules. Sustainability is further enhanced by the *Tetrahymena* Stock Center, which provides a dependable source of cells for the program. Although the initial workshops are somewhat costly, the subsequent potential for expanded access to the *Tetrahymena* modules using local teacher resources makes the overall program relatively cost efficient. Training local teachers in the use of *Tetrahymena* modules also provides a comparatively inexpensive mechanism for increasing and sustaining use of these educational tools in underfunded schools serving at-risk students. Providing support for workshop training for one expert teacher can ultimately facilitate the use of *Tetrahymena* in an entire school district, and impact large numbers of students.

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Figure 1. Bioinformatics Results

A) Schematic of functional domains in Photolyase (PHR1) obtained using ExPASy PROSITE (<http://prosite.expasy.org/>). *Saccharomyces* contains both photolyase signature domains 1 (orange) and 2 (blue) while the *Tetrahymena* homologs PHR1 and PHR2 contain only one photolyase signature domain. *Tetrahymena* PHR1 has signature domain 1 (orange) while *Tetrahymena* PHR2 has signature domain 2 (blue). B) UPGMA phylogenetic tree of various photolyase related proteins from various organisms. These trees help predict the degree of conservation in the protein and to what organisms it is most closely related (*data generated by Scott Kelsey and Archana Shrestha in BMS658: Recombinant DNA techniques, Biomedical Sciences, Missouri State University*). Phylogenetic tree analysis was performed using ClustalW (<http://www.genome.jp/tools/clustalw/>) and Mega 5.0 (<http://www.megasoftware.net/>).

Figure 2. Gene Expression Analysis by Reverse Transcriptase PCR (RT-PCR)

A) semi-quantitative RT-PCR of *DMC1* and *CYP1* (control gene) during conjugation (0–14 and 24 hours post mixing), vegetative growth (V), starvation for 18 hours (S), and genomic DNA control (G). Large changes in expression can be visualized on the gel. Here *DMC1* is highly expressed at 2–4 hrs and 12–13 hours during conjugation (*data generated by Emily Gallichotte, independent research, Keck Science Department, Claremont Colleges*). B) Relative gene expression of *THD13* (sirtuin-like deacetylase) determined using quantitative real-time RT-PCR. Data was obtained by dividing the *THD13* signal by that for *HHP1* and the numbers were set relative to the value for the logarithmically growing sample (LOG) being equal to 1.0. Various conditions can be analyzed such as starvation (STV) and treatment with 100 J/m² ultraviolet light and allowing recovery after the treatment (UV 0hr – UV 4hr). C) PCR amplification graph from the real-time PCR machine illustrates the amount of amplification that occurs for each condition. This raw data is saved in a excel file to yield the data that was used to generate the normalized relative *THD13* expression in panel B. D) Melt peak graph from the real-time PCR machine shows the purity of the products in each reaction. Large peak represents the cDNA product expected while the small peak represents some genomic DNA contamination (*data in panels B–D generated by Christopher Reynolds in BMS558: Recombinant DNA Techniques, Biomedical Sciences, Missouri State University*).

Figure 3. Localization of GFP and RFP tagged genes

A) GFP-HHP1 localized to chromatin bodies in the macronucleus (*data generated by Katerina Yale, thesis research, Keck Science Department, Claremont Colleges*). B) GFP-THD14 localized to the nucleoli around the edge of the macronucleus (*data generated by Allen Chen, thesis research, Keck Science Department, Claremont Colleges*). C) GFP-THD14 localized to the mitochondria in *Tetrahymena* (*data generated by Kristin Slade, Keck Science Department, Claremont Colleges*). D) GFP-DMC1 localized to the micronucleus in meiotic prophase (crescent phase) during conjugation at 3 hours (*data generated by Emily Gallichotte, thesis research, Keck Science Department, Claremont Colleges*). E) GFP-KDA3 and RFP-TCBP in conjugating *Tetrahymena*. F) GFP-KDA3 and RFP-TetrinA in conjugating *Tetrahymena*. G) GFP-KDC2 and RFP-TCBP in conjugating *Tetrahymena* (*data in panels E-G generated by students in BIOL 3492: Laboratory Experiments with Eukaryotic Microbes, Biology Department, Washington University*).

Figure 4. Coomassie stained SDS-PAGE of proteins interacting with FLAGHis₆-URM1
 Protein extract from an untagged strain, CU522 (5), and a strain containing a FLAGHis₆-URM1 epitope tag (U) were used in an immunoprecipitation with Anti-FLAG agarose beads. The beads were boiled with a non-denaturing loading buffer and the entire sample was loaded on a 4–18% SDS-PAGE. The gel was Coomassie stained and five bands were excised and sent for analysis by mass spectrometry (Doug Beussman, Chemistry Department, St. Olaf College). Bands in the URM1 immunoprecipitation lane yielded potential interacting proteins in all but band 5 and no proteins were identified in the control lane (5) by mass spectrometry (*data generated by Allie Maltzman and Kyle Cottrell in BMS558: Recombinant DNA Techniques, Biomedical Sciences, Missouri State University*).