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Cover image: Can you locate the ground squirrel in this picture? Photograph courtesy of Ethel Stanley.

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Bioscene: Journal of College Biology Teaching

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Deadlines for Submissions

April 1, 2005 for the May 2005 Issue

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Ramping Up to the Biology Workbench: A Multi-Stage Approach to Bioinformatics Education

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Abstract: In the process of designing and field-testing bioinformatics curriculum materials, we have adopted a three-stage, progressive model that emphasizes collaborative scientific inquiry. The elements of the model include: (1) context setting, (2) introduction to concepts, processes, and tools, and (3) development of competent use of technologically sophisticated tools. A curriculum involving the analysis of HIV sequence data is used to illustrate this framework and provide a context for discussing this student-centered, inquiry-based approach to bioinformatics education and literacy.

Keywords: bioinformatics, inquiry, HIV, phylogenetic trees, multiple sequence alignment

INTRODUCTION

The recent explosion of publicly available molecular sequence and structural data and on-line tools to analyze those data provide expanded opportunities to incorporate their use in undergraduate education. Although the technical requirement for accessing the databases and analytic tools is minimal -- browser-based Internet access -- engaging students in realistic biology problem solving is more complicated. The "ramping up" metaphor is used to describe a process in which conceptual understanding and tool usage are developed simultaneously and with progressive complexity. This approach allows us to emphasize the difference between the generally low *technical* barriers to manipulating analytic tools and the generally high *conceptual* barriers involved in the selection of appropriate data and tools, the interpretation of analytic results, and connecting the abstract molecular information to more familiar biological phenomena.

We have been involved in bioinformatics curriculum development projects aimed at bringing bioinformatics to several audiences including undergraduate biology majors, pre-service biology teachers, and teaching faculty. We have operated with the belief that meaningful learning can be promoted within carefully structured, deliberately ordered problem spaces that give students the opportunity to pursue research without becoming awash in the technical details of this dynamic and emerging field.

In this paper we present both a general approach to introducing bioinformatics problem solving and a specific instantiation of that general approach. We developed a notion of "progressive problem spaces" using a three-step approach to engage students (and faculty) in realistic research and problem solving using bioinformatics data and tools. These steps involve (1) establishing a context for the use of bioinformatics, (2) providing an introduction to the data, tools and reasoning patterns involved in bioinformatic analyses, and (3) creating open-ended opportunities for research using rich data resources and sophisticated analytic tools. This approach is exemplified with a set of activities that proceed from a basic orientation to bioinformatics to open-ended investigations of HIV evolution.

STAGE ONE

Context Setting: Seven Scenarios Activity

We developed an introductory context-setting activity we call "Seven Scenarios." Each of the seven scenarios in this collection (Parents, Police, Patents, Privacy, Patients, Profit, and Peanuts) consists of four or five short written statements, each written on a separate index card (Figure 1).

Seven Scenarios: A Context Setting Activity for Studying Bioinformatics & Biotechnology

Parents: expectant parents and a gene associated with a disabling condition.

1. Scientists have identified a gene and have developed a test for different forms of a gene.
2. One form of this gene is considered a risk factor for a disabling but not fatal condition.
3. A couple is expecting a baby.
4. Both prospective parents "carry" this form of the gene.
5. The parents are concerned about the costs of raising a child with a disability.

Police: culpability of someone accused of transmitting a virus.

1. A person is accused of sexually transmitting a virus
2. Police use blood tests to try to determine if that person is the source of the virus.
3. The virus causes a disease appearing years after the initial transmission.
4. The defendant's lawyer argued that because the viruses in the accused and the accuser were so different, her client should not be found guilty.

Patents: drug companies seeking gene patents.

1. A patent grants exclusive ownership of intellectual property so that the patent owner can profit from its use.
2. Many biotechnology firms are pursuing patents for gene sequences.
3. The companies hope that the gene sequences can be used to develop specific biological products.
4. There is currently a rush to apply for patents on any possibly useful sequences.

Privacy: a job candidate's pre-employment physical.

1. A candidate for a job is required to take a pre-employment physical.
2. Genetic analysis identifies a form of a gene that has been linked to high blood pressure.
3. The relationship between this gene form and high blood pressure is not well understood. Some people with the gene have normal blood pressure and many without the gene have high blood pressure.
4. The candidate is hired, but is told he will have to pay higher premiums for medical insurance.

Patients: a physician paying for genetic study of possible drugs to treat her patient.

1. A physician is treating a patient who has an aggressive and lethal cancer.
2. The physician pays a biotechnology company $37,000 to find potentially effective drugs.
3. The company identifies three drugs that are then used to treat the patient
4. The patient's cancer goes into remission.

Profit: for-profit and not-for-profit genomic enterprises.

1. The Human Genome Project is a consortium of academic research groups trying to determine the sequence of the human genome.
2. HGP (Human Genome Project) is a not-for-profit project that receives a great deal of public funding.
3. A for-profit company, Celera, uses the publicly available HGP data to check its work, fill in the gaps, and stay a step ahead.
4. Celera maintains a private database, available to corporations for subscription fees of 5 to 15 million dollars per year.

Peanuts: genetically modified organisms finding their way into the human diet.

1. A fast food restaurant recently had to dump some food because it contained an unapproved ingredient.
2. A strain of peanut has been engineered to resist a fungus known to wipe out a whole season's crop.
3. New foods do not need FDA approval if they meet three conditions: 1) the nutritional value is not lowered, 2) the food is already present in the human diet, and 3) the food is not an allergen.
4. Many people are allergic to peanuts.

Figure 1. *Scenarios used to establish a context for using bioinformatics.*

The cards can be distributed among the students, and groups are asked to assemble according to their scenario. Once the groups are assembled, each person in the small group reads his or her card to the group, and then the group discusses the scenario, considering these three questions:

- Is there any information about the scenario that you wish you had or that you felt was missing? In other words, was there enough information to consider?
- What issues (philosophical, historical, political, scientific, ethical) arise in discussion of this scenario?
- What kind of research or investigation would you consider doing based on this scenario?

After a few minutes of group discussion, the groups read their sentences to the other groups and report on the small group discussion, usually referring to the three questions. This activity usually generates discussions around people's own experiences with pre-employment physicals, their understandings of genetically modified organisms, and questions about how people pass along a serious disease. Often someone in the group has relevant specific knowledge or a personal experience to share. A variety of questions will undoubtedly arise and can be shared across the groups. It will be up to the teacher to decide if it is appropriate to attempt to answer some of these questions at this juncture or postpone them for later discussions.

There are several important outcomes of this short activity. First, we have learned much about what the students know and don't know about biotechnology and bioinformatics. Second, we have kindled their interest in these topics in part by reminding them of examples that they have read about in the news, heard about, or experienced. Third, we have involved everyone, from the beginning, in deliberately collaborative, low-stress, non-intimidating situations. By assessing background knowledge, sparking interest, and generating an experience and expectation of participation, we establish a context and a springboard for studying bioinformatics and engaging in bioinformatics activities.

STAGE TWO

Concept and Skill Establishment: Is He Guilty?

The second phase of our three-step progression provides opportunities for students to become more familiar with some of the types of data, techniques, and graphic representations that are used in sequence analysis. This activity involves working through a small problem in a series of three discrete steps to introduce learners to some of the ideas behind sequence analysis. The goal is to establish a shared conceptual understanding and introduce reasoning skills that can be applied to a variety of contexts and problems involving comparative sequence analysis.

In this example we examine the use of HIV sequence data as forensic evidence linking a Florida dentist and some of his HIV+ patients. Groups of students are provided with a series of printed materials, including raw data and the output from various bioinformatics analyses, and asked to determine whether there is evidence that the dentist is the source of HIV infection for his patients. By taking the analysis in several steps and allowing both small group and whole group discussion at each stage, it is possible to quickly bring the entire class to a relatively sophisticated understanding of how the analysis of molecular sequences can be used to support biological claims. The materials below provide an overview of our approach to establishing concepts and skills using the dentist HIV forensics example. The data files, images and additional discussion can be found on the web site <http://bioquest.org/bioinformatics/> and a similar activity is described in more detail in *Microbes Count!* (Donovan, 2003; Donovan & Weisstein, 2003).

Step 1: Sequence data

In the Spring of 1990 Kimberly, a 22 year old living in Fort Pierce, Florida, tested positive for HIV -- she had no identifiable risk factors for contracting the virus. Epidemiological research focused on an invasive dental procedure performed by an HIV+ dentist several years earlier. From the dentist's records a number of other HIV+ patients were identified, several of whom had no known risk factors for contracting the virus. The Centers for Disease Control and Prevention (CDC) became involved and the case received a great deal of media attention based on the public's concern that HIV+ health care workers may be a threat to their patients (Gentile, 1991; MMWR, 1990, 1991a, 1991b). Multiple lawsuits were filed by patients claiming that they were infected by the dentist and seeking damages. In court, attempts to link the patients' HIV to the dentist's HIV rested in part on comparative analyses of the virus sequences (Ou, et al., 1992).

In order to explore the role of sequence analysis in resolving this type of question, groups of students are provided with a small collection of raw amino acid sequence data from HIV viruses collected from three patients, the dentist, a local control and an outgroup (see Fig 2). We also distribute a printout of the abstract from a paper reporting on the analysis of these sequences, as well as one of the GenBank® sequence records (Ou, et al., 1992). These resources make the scenario more nearly "real" for students by allowing them to see the mechanisms used by scientists to share their data and report their results.

Dentist
NFTDNAKIIIVQLNASVEINCTRPNNNTRKGIHIGPGRAFYATGEIIGDIRQAHCNISREKWNNTLNQVVTELREQFGNKTITFNHSSGGDPEI

Patient E
NFTDNAKIIIVQLNASVEINCTRPNNNTRKGINIGPGRAFYATGGIIGDIRQAHCNISEEKWNNTLKQVVTKLREQFGNKTIIFNHSSGGDPEI

Patient F
NFMDNVKTIIVQLNESVQINCTRPNNNTRKSIHIAPGRAFYATGEIIGDIRQAHCNLSSIKWNDTLRQIAKKLKEQFGNKTIIFNQSSGGDPEI

Patient G
NFTDNAKIIIVQLNASVEINCTRPNNNTRRGIHIGPGRAFYATDRIVGDIRQAYCNISREKWNNTLKQVVAKLREQFVNKTIIFNHSSGGDPEI

Local Control
NFTDNTKTIIVQLNTSVTINCTRPGNNTRKSITMGPGKVFYAGEIIGDIRQAHCNLSRAAWNDTLKQIVGKLQEQFGNKTIIFNHSSGGDPEI

Outgroup
NFTNNAKTIIVQLKRPVKINCTRPNNNTRKSINIGPGRAWYTTEIIGDIRQAHCKINQTEWNNTLKEIVEKLREQFGNKTIQFKNHSGGDPEI

Figure 2. *Amino acid sequence data from HIV found in the dentist, three patients, a local control and an outgroup.*

As we progress through each of the sections of this activity, the driving question for groups to consider is whether they feel they have evidence to link the dentist to the HIV in any of these patients. For this section they are also asked to consider:

- What sorts of patterns do you see within/between these sequences?
- How are these sequences similar (different)? Are they all similar (different) in the same ways?
- How do you think this information could be used to determine if the dentist were the source of the HIV in the patients?

Groups are encouraged to keep a list of questions that arise during their discussion. Students often use highlighters or other visual methods to begin comparing the order of the letters in each sequence. They generally notice that the sequences have different lengths. Many also recognize that because the sequences are so similar, it is efficient to look for differences rather than similarities.

In the subsequent discussion of what the groups have learned, there should be an abundance of promising ideas and questions to address. There are often specific questions about how to interpret the data (e.g., What do the letters mean? What is a local control?). Other questions may focus on the nature of the virus (e.g., Are all HIV viruses identical within a person? How fast does HIV change? Do any two people have identical HIV viruses?). Still other questions might relate to making comparisons of the sequences (e.g., Is it significant that some letter combinations don't seem to change much and others change quite a bit? Are there likely to be more changes in one area of these sequences compared with other areas?). It is interesting to watch a shared language develop as students work to describe what the groups have seen. Some of these questions can be addressed directly, some are postponed, and others are reflected back to students to help them integrate their existing biological knowledge.

Step 2: Interpreting a multiple sequence alignment (MSA)

For the next round of group work, we introduce one of the standard techniques for comparing sequences, a multiple sequence alignment. This is, in many respects, what some students will have already begun to work on in their groups when presented with the raw sequence data. They are readily convinced that the longer the sequences and the more of them there are, the more cumbersome it becomes to align them by hand. They see that this is the perfect type of work for a computer. What is more difficult to have them understand, though, is that the parameters used in the algorithm for a sequence alignment reflect a set of assumptions about the relationships between those molecules. We distribute to the groups an alignment of the sequences with which they have been working, and provide them with information about the pairwise comparisons between sequences (Figures 3 & 4). Once again they work in their groups, and we prime their discussions with the following questions:

- Does the information presented in these outputs support the patterns you saw when you looked at the raw sequence data?
- Do you think some of the amino acid changes are more important than others?
- Why do you think that two of the sequences needed to have a "gap" (-) inserted to make them align with the others?
- How do you think this information could be used to determine if the dentist were the source of the HIV in the patients?

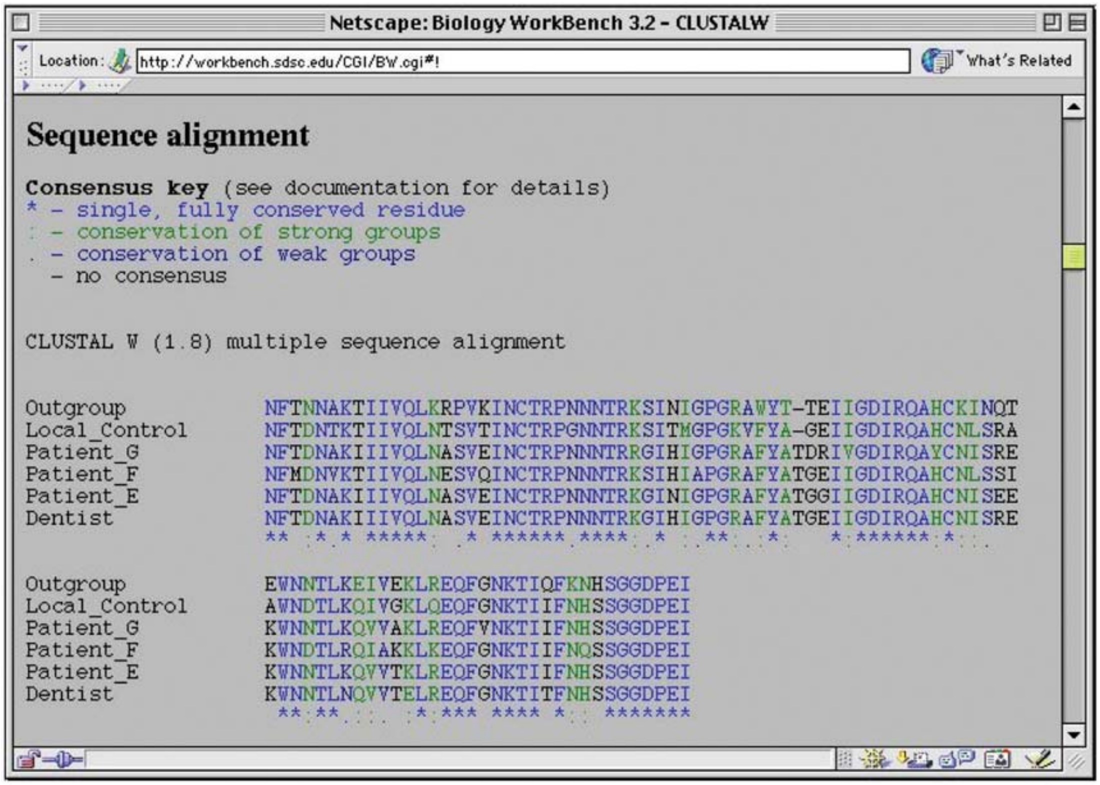

Figure 3. *A multiple sequence alignment of the HIV amino acid sequences listed in Figure 1.*

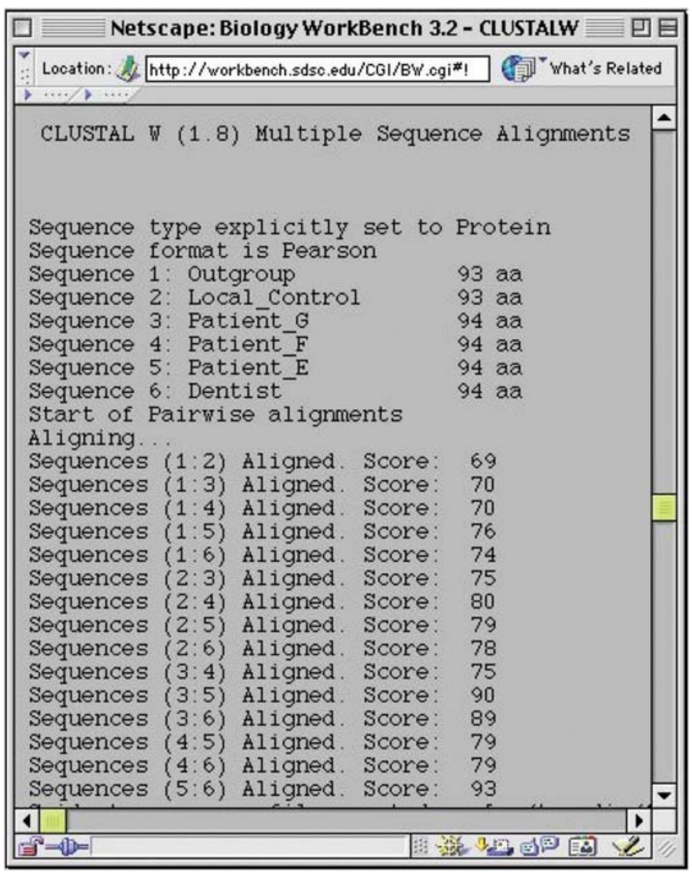

***Figure 4**. Pairwise sequence comparisons listing the percent identity between each pair of sequences from the sequences in Figure 1.*

The ensuing discussions provide great teachable moments. Groups that we have worked with have brought up the common origins of sequences as a source for their similarity, the notion of mutation "hot-spots," conservation of sequence for conservation of structure and function, and the similarities and differences between groups of amino acids. Still, even with MSA and pairwise comparisons, it is difficult for students to argue effectively for role of the dentist in transmitting HIV to certain patients.

Step 3: Reading trees

In the final step of this second-stage activity we provide each group with an unrooted tree built from their aligned sequence data (Figure 5). They then have another opportunity to work with their group to address the following questions:

- Does the information presented in this tree representation support the patterns you saw when you looked at the raw sequence data and the multiple sequence alignment?
- Why do you think some of the lines are longer than others? Do you think the places where the lines connect with one another is important? What does it mean?
- How do you think this information could be used to determine if the dentist were the source of the HIV in the patients?

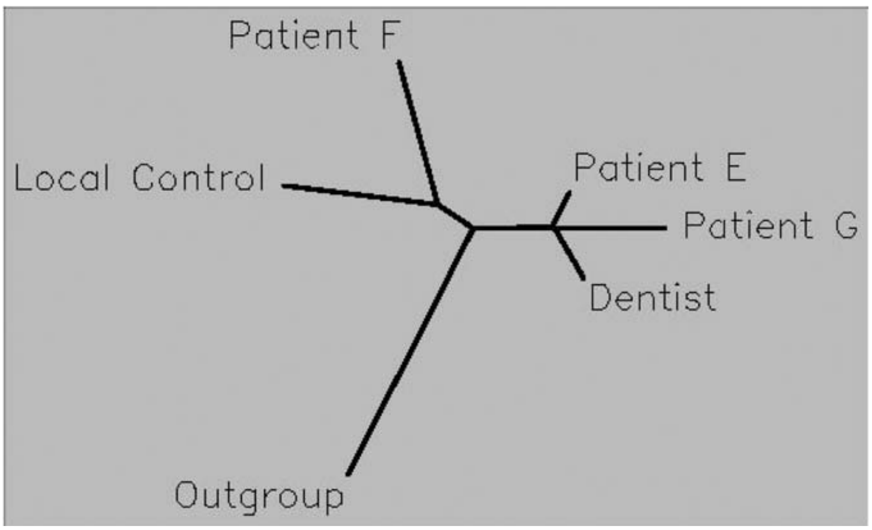

Figure 5. *An unrooted tree representing the genetic distance between the sequences in Figure 1.*

By the conclusion of this final discussion, we find that students have become conversant about a variety of important aspects of bioinformatics research including but not limited to: (1) the types of information that are associated with sequence data submitted to public research databases (2) ways to read similarities and differences between sequences from multiple representations of molecular data (3) how a multiple sequence alignment summarizes the comparisons of sequences (4) how a phylogenetic tree graphically represents the differences between sequences and can be used to develop hypotheses about evolutionary relationships (5) how evolutionary relationships between sequences can be used as forensic evidence.

STAGE THREE

Exploring HIV Evolution: An Opportunity to Do Your Own Research

The third stage of developing bioinformatics inquiry skills builds from the previous activity to engage students in investigations of their own questions using molecular data. This exercise is open, in that it provides students opportunities to make decisions and develop their own research strategies, but it is not unstructured. The use of a published data set both simplifies the problem, so students don't need to search for sequences or decide if particular sequences are appropriate to compare, and limits the range of questions that can be addressed, because the data set lends itself to certain types of analyses and is not appropriate for others.

This stage brings all of the pieces together. It involves students in, and connects them to, the biology, the analytical tools and a specific data set. We discuss the biology of HIV, emphasizing information that is pertinent to the data set they have been provided. We use a collection of several hundred sequences from 15 HIV+ patients taken over a period of time (Markham, et al., 1998). We hand out a summary table of the data that are available and discuss briefly how the data were collected (Figure 6). The groups are then asked to look over the summary data table for interesting patterns, and to think about possible research questions. As a whole class, we brainstorm possible research ideas, which accomplishs several teaching objectives. We get some concrete ideas in the air, further orient students to the data available, link the data to what we know about HIV biology, and illustrate the range of potentially fruitful investigations that one could undertake. At this time, we also call attention to the idea that the same data set can be used to generate multiple hypotheses.

Summary of the data set

Subjects: 15
Number of visits: 3-9
Number of clones per visit: 2-18
CD4 cell counts for each visit

Subject	Total Number of Visits	Total Number of Clones	Visit Number	Number of Clones	CD4 Count[1]
1	3	42	1	13	464
			2	16	305
			5	13	15
2	3[2]	24	1	6	715
			3	9	825
			4	9	830
3	5	39	1	4	819
			3	10	375
			4	9	265
			5	10	100
			6	6	45

***Figure 6**. Part of the data summary table for the Markham, et al. 1998 dataset.*

As a next step, we select one or two questions and model what the students will be asked to do in their own investigations. This allows us to work through the process of focusing a general question like, "Is there a particular change in the HIV sequence that causes the T-cell count to drop?" Together, we generate some specific analytic ideas that could be used to address this broad question. For example, one could compare sequences from individuals who did not have T-cell count drops to those who did, or maybe compare sequences from one time to those from a later time. These discussions help students recognize that a variety of decisions need to be made in order to make progress on any research question and that these decisions will be central to the process of relating their results to their scientific claims.

Next, the groups work together at their tables to begin defining their research questions and methods. Depending on the setting, we might introduce students to the Biology WorkBench website <http://workbench.sdsc.edu> to show them the mechanics of choosing sequences and running analyses. We build in multiple opportunities for feedback and peer review by getting groups to share their preliminary results with one another. The outputs the groups see are the same as the printed materials they worked with in the second stage. We encourage groups to print their findings and bring them back to the conference room, where they have table space to lay them out and consider the results in light of their research questions. This also promotes the important idea that the generation of computer outputs is only a preliminary step to answering their research question, which requires careful analysis and interpretation of those outputs, as well as a coherent synthesis and presentation of the investigation and results.

Students prepare posters and hold a research meeting at the end of this third stage. We generally see a high level of student engagement with each other's research. Having worked with the same data set and struggled with the same conceptual issues, the class has a chance to become a real research community.

DISCUSSION

At least five core aspects characterize our bioinformatics curricular model. First, the entire curriculum is set within an inquiry context, it is a question-based curriculum. Second, it is a collaborative model, in which students and teacher think and talk with one another in groups both large and small. Third, students are placed in a decision-making role; it is they who ask the questions, which they investigate. Fourth, the science and tools are taught in context. Finally, and this is where the "ramping up" occurs, there is an intentional progression of concepts and procedures from simple to complex, and from the more conceptual to the more technological. Complex concepts build on simple ones, and sophisticated technological tools are used to carry out tasks that are based on and emerge from the spectrum of concepts from the most basic to the most complex.

In our curriculum model, it is the students whose work is "up front" and the instructor whose work is "behind the scenes." That is not to say that we do not play an active or directorial role. We carefully construct biological scenarios that are interesting and that promote certain types of questions appropriate to the setting. We select molecular data sets that are rich with possibility. Our choices in designing these problem spaces are guided by four goals: (1) to establish real-world and science context, (2) to review and provide necessary biology content and relevant concepts, (3) to guide progressive exposure to and experience with bioinformatics data, techniques and representations, and (4) to develop an awareness of and facility with bioinformatics tools, such as Biology WorkBench, all in a context of inquiry. Over the course of the activities, we review our goals, and adjust our facilitation as necessary to accomplish them.

Understanding the uses of bioinformatics, what the various data represent, which tools to use, and what inferences are reasonable are essential to a successful bioinformatics educational experience. Conceptual, procedural and technological understandings are dynamic and fluid, and all must be present for meaningful learning and understanding in bioinformatics. Let us use our example to describe these three overlapping categories and some of the understandings within them.

Conceptual understanding means that students have robust knowledge that allows them to work with ideas in appropriate and meaningful ways. To be successful in bioinformatics, students need to be familiar with and understand large biological ideas such as inheritance, evolution, genetics, mutation and the somewhat more specific biological notions of DNA, transcription, translation, replication, amino acids, and protein synthesis, etc. More specialized bioinformatics concepts include knowledge of molecular databases and sequencing and other analytic heuristics and tools.

Procedural knowledge includes general scientific procedures, such as those associated with collaborative inquiry (Bruce & Levin, 1997) and problem solving (Peterson & Jungck 1988), but also specific procedures, such as multiple sequence alignment and analysis and gel electrophoresis. Interpretation of analytic outputs such as phylogenetic trees requires both conceptual and procedural knowledge.

Technological understandings likewise extend from the general to the specific, including the use of computers and basic applications for a variety of tasks, such as word processing and internet searching. Specific technological understandings include knowledge and skills associated with bioinformatics technology, both those using computers as a central tool, such as molecular database searching, sequence selection and retrieval, and subsequent analysis, as well as other tools such as wet lab apparatus. Use of bioinformatics technology can aid students in both the generation and interpretation of analytic outputs such as the phylogenetic trees mentioned above. Our bioinformatics curriculum provides experiences that highlight all of the components described above. The first stage, "Seven Scenarios," does not so much develop as elicit, assess, and lay groundwork for developing conceptual, procedural and technological understanding. It provides an opportunity for students to retrieve and demonstrate their existing conceptual knowledge. This also provides a form of global problem-solving procedural practice, as students work in groups to think and talk through possible problems to pose and pursue. Also through discussion, students set the stage for developing an awareness of technological possibilities.

In the second stage, students confront various representations of data, practice asking biological and procedural questions, and experience and develop fundamental technical knowledge of the basic processes on which bioinformatics is based, such as sequence comparison and analysis, tree building, and the interpretation of all of these. Their development of conceptual and procedural meaning allows them to develop an appreciation and see a need for using technology to investigate interesting biological questions.

In the third stage of our model, the students use, and develop skill with powerful and specific technological bioinformatics tools. They use these tools within an authentic research and content context; they use and build their conceptual and biology content knowledge, and they engage in real biological inquiry. Students are getting training in technology, as researchers, who can direct the technology to meet needs that *they* identify rather than as technicians who perform tasks set out by others.

Having constructed the scenarios and selected the data sets with the previously stated principles and goals in mind, we share them with students, and literally invite them to engage in discussion and inquiry. We provide a flexible structure in which to discuss and inquire, but the students are decision makers at each stage. In the first stage, students evaluate the information they have been given and they decide what else they need to know. In the second and third stages, students pose and pursue questions of and with their data, progressively applying and enhancing conceptual, procedural and technological knowledge as they pursue their investigations, making and reflecting on their research decisions as they proceed.

The curriculum model we have designed is inquiry-based, collaborative, student-centered, and intentional in both sequence and context. In their use of technologically sophisticated tools that rapidly carry out familiar procedures based on understood concepts, collaboratively investigating answers to interesting questions they themselves have set, students develop rich and contextual knowledge and understanding of biology and bioinformatics concepts, procedures, and technologies.

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Manuscript Guidelines for *Bioscene: Journal of College Science Teaching*

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Manuscripts submitted to the Bioscene should focus primarily on the teaching of undergraduate biology or the activities of the ACUBE organization. Short articles (500-1000 words) such as introducing educational resources provided by another organization, reviews of new evolution software, suggestions for improving sampling methods in a field activity, and other topics are welcome as well as longer articles (1000-5000 words) providing more in depth description, analyses, and conclusions for topics such as introducing case-based learning in large lectures, integrating history and philosophy of science perspectives into courses or initiating student problem solving in bioinformatics.

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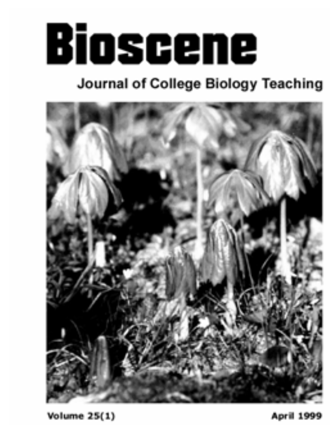

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Tapping Recent Alumni for the Development of Cutting-Edge, Investigative Teaching Laboratory Experiments

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ABSTRACT: This project presents a model for the development of an innovative, highly-experimental teaching laboratory course that centers upon collaborative efforts between recent alumni currently enrolled in Ph. D. programs (consultants) and current faculty. Because these consultants are involved in cutting-edge research, their combined talents represent a much wider range of expertise than any individual faculty member could bring to teaching laboratory development. Furthermore, the consultants' understanding of the context of the institution and its curriculum uniquely qualifies them to serve in this capacity. In this particular project, this model was applied to laboratory course development for Biology 308, a course in cellular vertebrate physiology. Consultants were selected who were involved in research in the areas of renal cell physiology, cell motility, hormonal signaling, immunology, and neurology. Each consultant's experimental system was used to develop a teaching exercise that investigates topics relevant to the course. These are areas covered in Biology 308 that were either not previously represented in the laboratory course, or the original exercise in these areas was highly routine and unengaging. The project has significantly increased the breadth of expertise at Knox. In addition, course evaluations have indicated that the students find the lab course more interesting and significant. Students are more thoroughly engaged with the exercises because they feel they have a greater stake in their outcome.

KEYWORDS: graduate student-undergraduate student interaction, investigative teaching labs, sodium/potassium ATPase α-subunit, teaching laboratory course development, undergraduate

INTRODUCTION

Given the limited size of small liberal arts colleges, faculties are often asked to teach classes outside of their formal training so that a department can cover its discipline. At Knox we have a biology department of only five faculty members. Though I am a plant cell and molecular biologist, I was asked to teach an upper-level course in vertebrate physiology with a decidedly cellular focus. The course had a legacy of being very popular with our majors, and it was seen as essential for students pursuing graduate or medical school. With prerequisites of three introductory biology courses in ecology and evolution, organismal form and function, and cell and molecular biology and with introductory chemistry strongly recommended, it was mostly populated by juniors and seniors. The course I initially developed and taught in my first years at Knox took a traditional lecture-and-lab approach. I felt much more comfortable in the classroom, because the lecture portion covered material well within the grasp of any respectable biologist, but the laboratory section was an altogether different challenge. In constructing a lab experience, I dug through countless lab manuals, and after making what I felt were necessary modifications and improvements, I had what I thought was a set of lab experiences that supported the lecture well. But as I taught the course, I became greatly frustrated by how superficial the experiences seemed to be. For students the next requirement in the biology major after Vertebrate Cellular Physiology is independent research, and the experiences obtained in my teaching lab were not preparing students as well as they might to make the most of an opportunity to work independently. The biggest problem was that the laboratory section asked the students to do exercises rather than experiments. I knew well that the best learning would result from open-ended experimentation (National Science Foundation, 1996; Rothman and Narum, 1999). Furthermore, I did not feel invested in the laboratory course. In my other courses, I had developed the exercises from my own expertise in the field. This could not be easily accomplished in this course; I simply did not have the training.

It was a conversation with a former student of mine, then enrolled in a Ph. D. program in cell biology, that got me thinking about an answer. After hanging up the phone from our conversation, I found myself wishing there was some way to get her to help me develop a lab based on the work she was now doing in hormonal signaling. From here, I made a list of other former students enrolled in Ph. D. programs in fields encompassed by the course. I got in touch with each of them about the work they were doing and asked them if they might be interested in working with me to use their experimental systems to develop cutting-edge investigative teaching experiences for the Vertebrate Cellular Physiology course. Recruiting former Knox students would help insure that the laboratories were appropriately challenging yet "do-able" at Knox. The National Science Foundation (NSF) had just announced a new Leadership Project under their Improvement in Laboratory Instruction Program that was an ideal funding source for the project (this program is currently known as Curriculum, Course and Laboratory Improvement). Eight months later I had NSF funding and the project was under way.

Details of the Approach

Five Knox alumni (graduate student consultants) and their Ph. D. thesis advisors signed onto the project. I visited each of their research laboratories to see first-hand how their experimental system worked. This visit also gave us an opportunity to lay out preliminary ideas for teaching laboratories. For two to three months following each visit, the consultant and I kept in close contact via phone, e-mail and fax. We brainstormed through several possible teaching experiments, with the goal of focusing our efforts on the one with the best potential for success. We also sometimes bounced ideas off of the thesis advisors. The first requirement was that the exercise had to be investigative. It was also important that the experiment was structured so that it made full use of the scheduled laboratory period, which is 150 minutes in duration; though returning to the laboratory outside of formal meeting times was possible, we tried to keep "off hours" at a minimum. To insure the experiments could be sustained once the grant expired, we also worked to keep costs down as much as possible. Also, we put a premium on experiments that approximated, as much as possible, the real excitement of the research laboratory. We looked for experiments that utilized research instrumentation available in the department and gave the students a sense of investment in their work and challenged them to bring concepts they were learning in class to the research bench.

Once focused on the candidate experiment, we developed a step-by-step protocol from which we made a list of the necessary material and equipment. We also developed a bibliography, including references for background, methods, and general principles. After we had worked through the logistics, and the supplies and equipment were secured, the graduate student consultants visited our campus to run through the experiments on a trial basis. We made notes about any errors or lack of clarity in the protocol, including potential bottlenecks where students may likely make a mistake, and what the "pre-lab" lecture would have to emphasize. Finally, we discussed how student performance in the laboratory would be evaluated.

A total of five exercises were developed, covering hormonal signaling through the G-protein cascade, interferon in the immune response, actin-based cell movement, nerve growth factor (NGF) in neuron-neuron synapse formation, and Na^+/K^+ ATPase expression and nutrient uptake in nephrons. The exercises were two to three laboratory periods in duration (i.e., 2 to 3 weeks). In some cases an exercise was initiated the same day that another exercise ended. In addition to time spent in the scheduled laboratory period, most of the experiments did require some time outside of class. Most of this time was spent on growing, maintaining, and manipulating cell cultures. The NSF grant provided funding to equip a tissue culture facility (The Center for Cell and Tissue Culture) that is located directly adjacent to the teaching laboratory. It also provided funding for site visits and modest stipends for the project participants as well as supplies for the first two years. The total cost for running the labs was estimated at $165 per student.

An Example Laboratory

The experiments conducted on Na^+/K^+ ATPase expression and nutrient uptake in nephrons are presented as an example of the sort of laboratory experiments we developed and how the students were engaged in the laboratory. This exercise was introduced with assigned readings on the expression of the alpha subunit of the Na^+/K^+ ATPase (α-SU) in Maiden-Darbey Canine Kidney (MDCK) cells and its dependence on Ca^{2+} for the establishment of the cadherens junctions necessary for establishing and maintaining cell polarity in epithelial tissues (Cantley, 1981). The first reading(s) in each unit was discussed as a class, setting an example for how to approach and analyze a paper. It also provided an opportunity to begin building a model of how the system operated, which became a model, in turn, for talking about potential experiments. Following this, the students read two additional papers in the field (Caplan *et al*., 1986; Hammerton *et al*., 1991). They were encouraged to discuss these papers among themselves. Our next discussion entailed adding details from their readings to our working model and then identifying reasonable questions that could be asked (Fig. 1). In this particular case, the students had read that the polarized expression of α -SU could be established within 24 h of cell confluence if Ca^{2+} were present; without Ca^{2+}, polarized expression could be established in cultured cells 18 h following its addition. They asked many good questions with solid rationale for asking them. It was then important to help them realize that there are constraints about what types of experiments can be done in science to answer those questions (and that at Knox there are even more constraints) (Fig. 1). To obtain answers to the questions, the students identified the sort of experiment that would have to be done. Through this process we determined that as a class we would investigate what happens to the expression of α - SU when confluent cells grown in Ca^{2+}-containing medium are shifted to Ca^{2+}-free medium (with EGTA). This process was not left completely to chance. The papers the graduate student consultants and I selected for the students to read were chosen to point to fairly obvious experiments that could easily be done at Knox. In addition, I did play a role in steering the discussion toward the most feasible investigations.

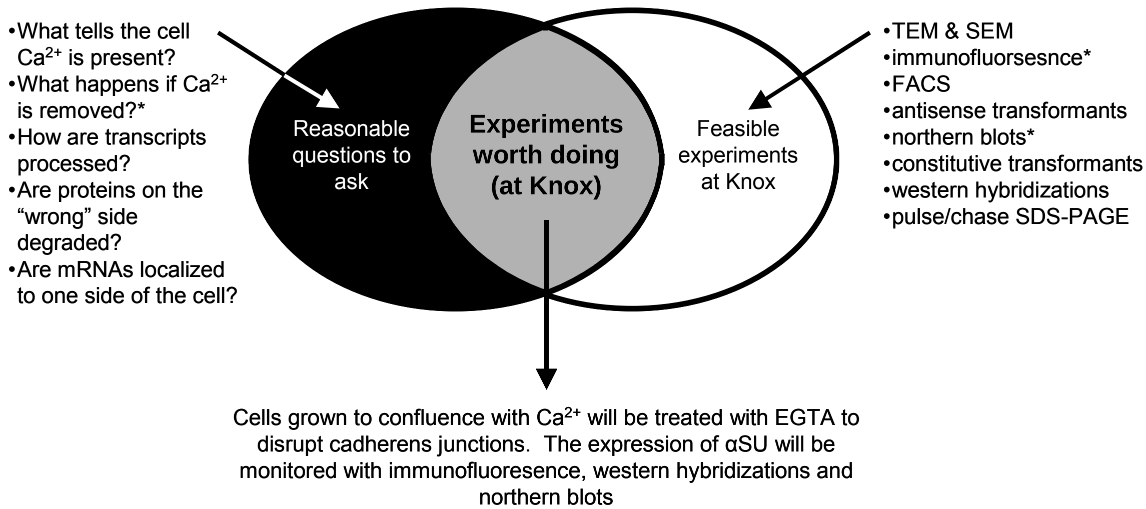

Figure 1. *Process for focusing class discussion to a workable experiment. Discussions generated a list of worthwhile experimental questions as well as a list of feasible experimental approaches to answer those questions. Where there was overlap, students chose a specific experiment, then worked out the experimental details. *Indicate experiments easily done at Knox.*

Once the students agreed upon the general experiment, class discussion turned to the practical issue of defining experimental and control treatments. The students constructed a timetable for organizing their work. Students worked in pairs, and each pair of students had responsibility for obtaining data for a given sample of the overall experiment. This approach highlighted the collaborative nature of science, and it helped to make each student feel invested in the experiment. Each pair of students ran the sample in duplicate, with each student in the pair responsible for one of the replicates. Running samples in duplicate insured that each student had an opportunity to manipulate a sample, and it also served to provide confirmatory or back-up data. In addition, I prepared cells to have at-the-ready should there be a problem with a given sample. It was important that in the end we had a complete set of interpretable data.

During the remainder of the laboratory period, the students began growing their cells and preparing the solutions they would need for executing the experiment. Each group was given a flask of MDCK cells, which, over the course of a week, they would on their own time, split into three sub-samples and grow to confluence (T-75 flasks for northern blots, T-25 flasks for western hybridizations, and 6-well plates with coverslips for immunofluorescence). At confluence, the students came in to switch their cells to Ca^{2+}-free medium for the designated time interval of their assigned sample. The timing of the switch was such that all of the samples were ready for harvesting during the laboratory period of the following week.

After this, the students executed their own RNA and protein extractions and prepared their cells for immunofluorescence. The electrophoresis was begun during the laboratory period, but was terminated in the evening. The RNA and protein in the electrophoretic gels were electroblotted to nitrocellulose hybridization membranes overnight, and non-isotopic detection of RNA and protein on the blots was done the following days during the normal lecture period. Students signed up for time slots to use the fluorescence microscopes. Photographs of northerns, westerns, and immunofluorescence images were acquired digitally and posted on the class web site so that everyone in the class would have access to all the results.

The final step involved writing a paper describing their findings. The students had five samples in their northern hybridizations (Fig. 2A), western blots (Fig. 2B), and immunofluorescence images (Fig 3). With this rich set of data, we discussed their results as a class before the students began writing their co-authored papers. They could see that the removal of Ca^{2+} affected protein levels (Fig. 2B) and protein distribution (Fig. 3) but not mRNA levels (Fig. 2A). Furthermore, they could see that after protein distribution was affected (within the first 4 hours), the levels of proteins in the cell decreased. Each student of each pair independently wrote drafts for the introduction, materials and methods, results, and discussion sections. They then were required to critique each other's drafts (within the pair), making detailed and helpful comments on what was done well and what needed improvement. They then collaborated on writing all the sections of the report, building upon what they had originally drafted. The final manuscript was written according to the instructions for authors for the *Journal of Cell Biology*, including the preparation of figures and tables. They handed in their final manuscripts and their drafts so that I could assess the contributions and progress made by each student. The graduate student consultants were in contact with the students via e-mail during the experiments as a resource for problem solving and discussing interpretations, etc. In some cases they also contributed to the evaluation of the final reports.

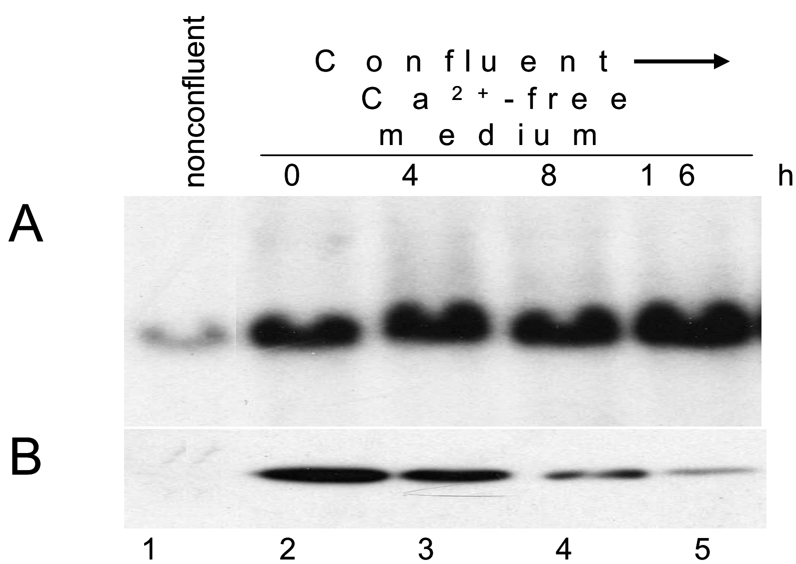

***Figure 2**. Student northern and western blot data examining the effect of Ca^{2+} removal on the expression of the α-subunit of the Na^+/K^+ ATPase (α-SU) of confluent MDCK cells. A) Transcript levels were analyzed by northern blots. B) Protein levels were analyzed by western blots. Cells were either grown to subconfluence (lane 1) or confluence followed by incubation in Ca^{2+}-free medium for 0 (lane 2), 4 h (lane 3), 8 h (lane 4) or 16 h (lane 5) according to the procedures of Grindstaff et al. (1996). RNA was isolated from cells grown in T-75 flasks using the TRI Reagent method according to manufacturer's instructions (Molecular Research Center, Cincinnati, OH) and quantified using A_{260}. Twenty μg of total RNA per sample were electrophoresed into 1.2% agarose gels (Maniatis et al., 1982) and capillary blotted to Gene Screen Plus membranes (New England Nuclear, Boston, MA). Northern blots were probed according to the procedures of Church and Gilbert (1984) by Stratagene's (La Jolla, CA) Illuminator chemiluminescent method. Proteins were extracted from cells scraped from the bottoms of T-25 flasks and homogenized in 1-mL glass tissue homogenizers. Thirty-five μg of total protein per sample were separated by 12% SDS-PAGE and electroblotted onto nitrocellulose following BioRad (Hercules, CA) Mini Protean instructions. α-SU was detected using Amersham's (Piscataway, NJ) ECL western blotting system with anti-α-SU antibodies (Grindstaff et al., 1996). Confluence increased α-SU mRNA levels, and protein accumulated. Shifting to a Ca^{2+}-free medium progressively diminished α-SU protein levels, though its mRNA levels remained high.*

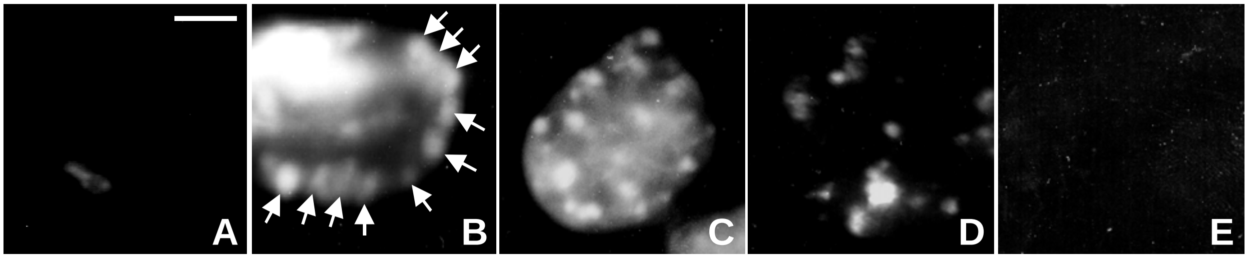

***Figure 3**. Student immunofluorescence images showing the effect of Ca^{2+} removal on the distribution of Na^{+}/K^{+} ATPase α-subunit in confluent MDCK cells. Cells were grown according to the procedures of Grindstaff et al. (1996) to A) approximately 80% confluence (nonconfluent control sample), B) confluence, C) confluence then shifting to Ca^{2+}-free medium for 4 h, D) 8 h, and E) 16 h. Cells were grown on coverslips in 6-well plates and then fixed and immunofluorescently labeled according to the procedures of Welch and Suhan (1986) using anti-α-SU antibodies (Grindstaff et al., 1996). Confluence resulted in α-SU protein localization to basolateral membranes (arrows) (bright fluorescence in upper left is noise also visible under normal light). Shifting to a Ca^{2+}-free medium rapidly delocalized α-SU from its basolateral membrane localization and overall signal diminished rapidly. Bar = 25 μM. Micrographs are at equivalent magnifications.*

Following the discussion of the results, we began the next set of experiments in the second half of the laboratory period with discussions of the next paper and the initiation of cell cultures. In each successive laboratory, the discussions progressed more quickly to the identification of a research question and the initiation of experiments. In some years, the questions evolved from the results of investigations conducted by students in previous years. In some years after learning what the students before them had done, questions evolved from the results of earlier investigations done in the class.

OUTCOMES

At this point, ten years after the start of the project, the exercises I developed with the graduate student consultants have each been used several times. Knox evaluates each lecture and laboratory course with a standard course evaluation form. In addition, I have developed a more open-ended qualitative survey to gain more critical insights. The student responses on the standard Knox evaluation forms showed marked improvement for the laboratory section (Table 1). In general, questions relating to the impact of the course and how challenging or stimulating it was, and the overall quality of the laboratory, showed substantial increases, yet there were not substantial differences in organization, preparation or effectiveness on my part. My original intent had been to have the students do all five exercises in each year. However, it became apparent in the first year that this was not feasible, and student feedback on my own evaluation forms indicated that the laboratory course was requiring too much work and time. This was especially the case when it came to writing up the results of one lab while starting the next; there simply was not enough time devoted to writing. I modified the course and used three to four exercises each year during the 10-week quarter. Each laboratory experiment is intensive, and students are learning critical thinking skills that require time for development. What is sacrificed with regard to content, I feel is more than compensated for by an in-depth understanding of investigative process and by a more in-depth understanding of the research problem (National Research Council, 1997). Students are better prepared to do well in their independent research projects. The techniques they learned were immediately applicable to the research programs directed by colleagues in Biology and Biochemistry. The Center for Cell and Tissue Culture is available for use by research students and faculty whenever it is not reserved for course use.

Although each pair of students ran duplicate samples and I had prepared back up material in the event of cell culture contamination, there were occasions when a “data hole” resulted because a group failed to process a sample correctly. Fortunately, in most years, enrollments in the course were such that two groups processed at least some of the key experimental or control samples. In spite of this, for most of the experiments done over the years, at least one of the samples had some sort of problem even in the very best of the various replicates (e. g., Fig. 3B). There were relatively few years, however, when all replicates of a given sample were absolutely unusable, and in such events it was often possible to “borrow” data from previous years. A full set of data was important in allowing the students to learn the most from their writing experience.

Table 1. *Results from student evaluations using Knox College's standard evaluation forms. Only questions in the laboratory section of the Knox College course evaluation form are shown. For 1987-1991, N = 98; for 1992-1999, N = 142*

Statement	% Responding Strongly Agree / Agree / Disagree / Strongly Disagree	
	1987-1991 Average	1992-1999 Average
This laboratory made a significant contribution to my education	13 / 52 / 24 / 11	45 / 42 / 6 / 7
For my preparation and ability this laboratory was appropriately demanding of my intelligence	23 / 57 / 17 / 3	63 / 34 / 0 / 3
This laboratory stimulated my personal initiative to thought and learning	10 / 45 / 31 / 14	51 / 43 / 6 / 0
Generally, the instructor was well prepared for the lab	86 / 11 / 3 / 0	83 / 12 / 5 / 0
This laboratory was well organized	78 / 18 / 2 / 2	73 / 22 / 2 / 3
Criterion	**% Responding Excellent / Good / Satisfactory / Fair / Poor**	
	1987-1991 Average	**1992-1999 Average**
The overall quality of the laboratory	29 / 34 / 30 / 7 / 0	73 / 14 / 13 / 0 / 0
The effectiveness of the instructor	78 / 20 / 2 / 0 / 0	82 / 18 / 0 / 0 / 0

In addition to the problem of data holes, other technical difficulties cropped up from year to year. For example, in the case of the sample lab exercise presented in this present paper, Ca^{2+} removal caused the cells to separate and lift off from the coverslips, making it difficult for the students to obtain quality micrographs. This was partially remedied by using poly-lysine-coated slides. Although frustrating, these sorts of unanticipated technical difficulties provided opportunities to build problem-solving skills. They also presented a more realistic view of the obstacles inherent in research.

In addition to the laboratory exercise developed specifically for the upper-level Vertebrate Cellular Physiology course, the brainstorming process we went through in the early stages of the laboratory development process generated several interesting "spin-off" exercises that were appropriate for the introductory Cell Biology course. For example, the MDCK cell lines were grown to confluence on filters to monitor the rate of vectoral transport of candidate solutes (amino acids, proteins, disaccharides, monosaccharides, urea, etc.) across a polarized epithelium. Students could investigate a wide range of substrate-containing solutions and incubation conditions. These experiments helped to enrich the introductory laboratory sections by introducing new investigative exercises.

As the exercise became dated over time, I obtained internal funds to bring in other alumni to develop teaching exercises using the same approach. These later exercises had some funding constraints that limited what sort of equipment could be used in the project, but some modest equipment purchases were still possible. Instead of bringing in a fleet of alumni, these exercises were developed at the more modest rate of one every year or two. Recently an alumna of the course became a graduate student consultant.

For the Vertebrate Cell Physiology students, the laboratory experience was empowering. The experiments in which they were engaged had clear links to ongoing research. Feedback on my evaluation forms indicated the students greatly appreciated this. These links were underscored by the fact that their lab work was based on work from a research lab in which a Knox College graduate was pursuing cutting-edge research. I know that, for many students, this was an important indicator to them about their own potential, especially for students who were not performing as well in the lecture section. Furthermore, the experimental component in each laboratory exercise helped to diminish the perception that the student must correctly come up with some answer already known to the teacher. This small but significant shifting of the power base helped the students begin the process of imagining themselves as investigative scientists. The work in the classroom began to feel more like that of a research team. Finally, students felt far more invested in their writing. They had ownership of original data, and student comments indicated that it was, “the first time [they] really cared about writing really well.”

Indeed, the quality of the student writing was significantly improved from what had been before the course had been redesigned. Students commented that they learned from their lab partners as they critiqued one another’s drafts. In addition, students felt that the collaboration required for the final paper was very beneficial to honing writing skills. They also found it useful to follow real-world instructions to authors for manuscript preparation. However, some student pairs did not work well together. In some cases this was due to different writing abilities between the group members. I found this could often be mitigated if I arranged for a stronger writer (not necessarily the partner) to help the weaker writer produce a better draft. In such cases I made certain I could identify a particular strength in the weaker writer that I could call upon later. This was all done discretely, but for the students involved it helped them to see that they each brought their own strengths to the research. In other cases it was a problem of one member of a group being too dominant in the partnership. I allowed students to swap partners from one experiment to the next, and I found the students to be fairly good at self-sorting. However, with some students I needed to intervene more directly and ask them to give their partner a chance to participate more fully in the science.

The graduate student consultants also gained some insights from the project. First of all, because many of the experiments were “what if?” experiments that would have otherwise been too risky for the graduate student to invest much time, there were occasions when the graduate students used the data generated by the class as preliminary data for subsequent follow up experiments when the class results looked promising. In addition, the graduate students gained unique insights on the connection between teaching and scholarship and why scholarship is valued at primarily teaching institutions, as well as why quality laboratory instruction might be important at research institutions (National Research Council, 1997). Graduate student training typically develops a sense of teaching and research as opposing obligations competing for limited time. This sense is reinforced by what they hear from their research mentors as their mentors complain about teaching “loads.” For graduate students fellowships provide research “opportunities,” while assistantships come with teaching “obligations.” As a result, the impression is set early on in the life of a prospective faculty member that the teaching laboratory is a low priority chore distinct from and at odds with research interests. For the students involved in this project, they developed a better appreciation for the synergy between research and teaching.

Bringing back recent graduates who have gone onto Ph. D. programs in the sciences can serve as a mechanism for invigorating teaching laboratories and keeping the laboratory experience relevant and more experimentally-driven in virtually any science or engineering discipline. Our former students are exposed to up-to-the-minute research, and they represent a broad, excellent and untapped source of expertise for keeping undergraduate laboratories current and exciting.

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In Memoriam -- George O'Connor

A long-time, sporadic, member of ACUBE (going back to AMCBT) died on December 17, 2004. George O'Connor was a Professor of Biology at Rockhurst University for 35 years. He was 64 years old and looking to retire in two years. His on again, off again relationship with ACUBE began in 1969 when he came to Rockhurst, and was interrupted as his family grew in number. He was very devoted to his family and they took precedence over many aspects of his life. He taught Invertebrate Biology, an offshoot of his love of raising tropical fish, as well as a means of working his way through college. He also taught General Biology, Anatomy and Physiology, and in later years added Research Techniques and Evolution to his responsibilities. In the past few years George with John Koelzer of the Mathematics Department at Rockhurst developed a course in Mathematics of Biology. They presented a paper on this course to ACUBE at the last meeting. Since his children moved onto their own careers he was planning to continue and expand his contacts with his ACUBE friends. He was a good friend and colleague, and will be missed by many.

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Teaching Population Growth Using Cultures of Vinegar Eels, *Turbatrix aceti* (Nematoda)

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***Abstract*:** A simple laboratory exercise is presented that follows the population growth of the common vinegar eel, *Turbatrix aceti* (Nematoda), in a microcosm using a simple culture medium. It lends itself to an exercise in a single semester course.

Key words: ecology, invertebrate, little-r, population dynamics, vinegar eel

INTRODUCTION

The dynamics of population growth is a challenging topic to explore in an ecology course that is only one semester in length. Two uncomplicated options for instruction include analyzing extant data sets (Frazer, 1991) and use of computer models (Alstad, 2003; Donovan and Welden, 2002; EcoBreaker© 2004; Jungck et al., 2003). Fast-growing microbes such as bacteria, yeasts, algae, and protists, or other organisms such as fruitflies and *Lemna* (Beiswenger, 1993; Gause, 1934) also can be employed. Unfortunately, exercises based on those models are more time consuming and add the possibility of failure. This paper describes a simple, inexpensive laboratory exercise that follows population growth of the vinegar eel (*Turbatrix aceti*; Nematoda) in microcosms using a simple culture medium comprising 1.5 L of pasteurized apple cider vinegar, and a bit of decaying apple.

Goals: Students will explore population growth and the constraints that limit it. This will be accomplished by studying the growth of a population of the vinegar eel (*Turbatrix aceti*) in an environment with limited resources.

Skills: A small culture of *T. aceti* has been started. Students will follow simple instructions to collect and record the data of population growth. They will perform simple calculations to determine *little-r* for the experimental population. Students will learn the operation of the Millipore® filter apparatus.

Learning Outcomes: The basic principles of hypothesis formulation, experimentation, data collection, analysis, and presentation, as they relate to population growth, will be reinforced.

MATERIAL AND METHODS

Cultures of *T. aceti* are available from several biological supply houses. These nematodes are easy to grow and the cultures last for years with little maintenance. A culture may be started by placing ca. 1.5 L of pasteurized vinegar, two pieces of apple (ca. 2 x 2 x 4 cm), and a small aliquot (≈2 ml) of an active *T. aceti* culture into a 2-L flask, closed with a 1- or 2-hole rubber stopper to permit some gas exchange. The population growth experiment reported here ran for 13 weeks. Thus, to fit this time frame into a semester, the culture was established three weeks before the start of the fall semester. Once a week, 3 replicates of 1-ml each were removed and the number of animals per ml of culture fluid was determined using membrane filter technology (see below). However, the population density also could be determined by other direct count methods such as using a Sedgewick Rafter counting cell (Wildlife Supply Co., Saginaw, MI) or a plankton counting chamber (Canimpex Enterprises Ltd., Halifax, Nova Scotia, Canada). In practice, the latter two methods should be less expensive then using membrane filters. Also the animals are observed directly, so the difficultly in deciding whether an object is a live *T. aceti* or is debris (e.g., dead animals and molted cuticles) is much less difficult. I used the membrane filter technique because I wanted the students to learn that technique.

Replicate samples from the *T. aceti* culture were taken using the following protocol. The culture was mixed by swirling the flask for about 15 sec. before a 1 ml aliquot was removed from the upper 5 cm using an automatic pipette. Each of these samples was filtered using Millipore® tower assembly and a 25 mm, 1.2 µm pore size, gridded filter (Figure 1), with a vacuum of <1 atm. (NB: Gridded filters are more costly, but aid when the animals are counted. Filters with larger pore sizes would probably be better as a small pore size captures more debris. Before filtration the membrane filters can be labeled along their outer edge using a ballpoint pen.) After filtration, the filters were removed from the tower assembly using flat forceps, placed over a 22 mm hole on a plastic drying rack, and weighted down with ca. 23 mm ring weight to hold the filters flat while drying. Filters were allowed to dry for 24–48 hrs. before examination. Whereas all the samples could be saved for a laboratory session later in the semester, in the results reported here, the students processed the samples within one week.

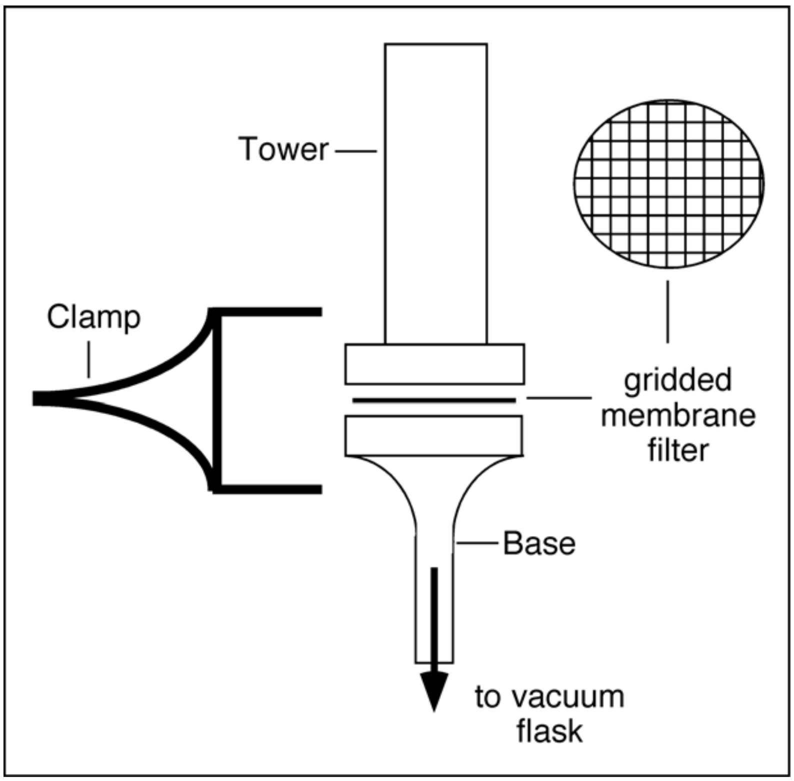

Figure. 1. *Schematic view of a Millipore filter apparatus.*

While all of the results discussed here were done without staining the *T. aceti*, one student tested various concentrations of four stains in an attempt to improve visibility of the *T. acet*i on the filter. The stains that were tested were Rose Bengal (at 0.01, 0.05, and 1%), Methylene Blue (0.3%), and Nigrosin (for the negative stain in bacteriology). The best results were obtained by adding one drop of Nigrosin per 10 mL of culture fluid for 5–10 mins. before filtration. Thus, these staining techniques require the removal of larger volumes of the culture fluid.

To examine the filters for *T. aceti*, 1–2 small drops of immersion oil were placed on a clean microscope slide and, using flat forceps, a dry filter was positioned over the drop of oil so that the grid lines of the filter paper are aligned as shown in Figure 2. Then a second drop of immersion oil was put on the top of the filter, a coverglass was placed on the filter, and gentle pressure was applied to the coverglass (Figure 3). This step distributed the oil over the filter and eliminated most of the air bubbles. Excessive pressure can crack the coverglass. (NB: The outcome of this is that the filter paper becomes clear. At this point I ask the students to consider the physics of this interesting optical phenomenon.) The last step before examining the filters for *T. aceti* is to have the students wipe off any oil that may have gotten onto the bottom of the slide.

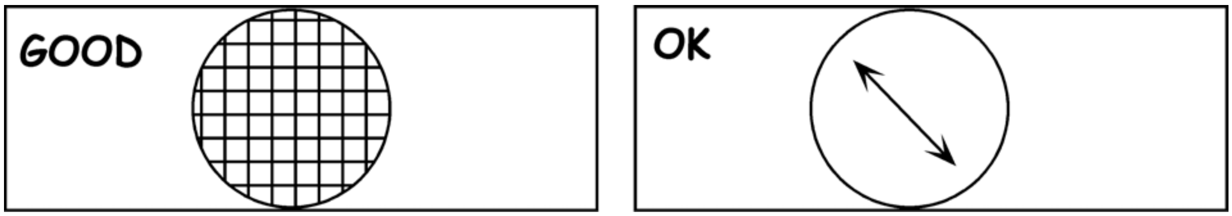

Figure. 2. *Convenient (left) & inconvenient (right) positioning of a filter on a microscope slide.*

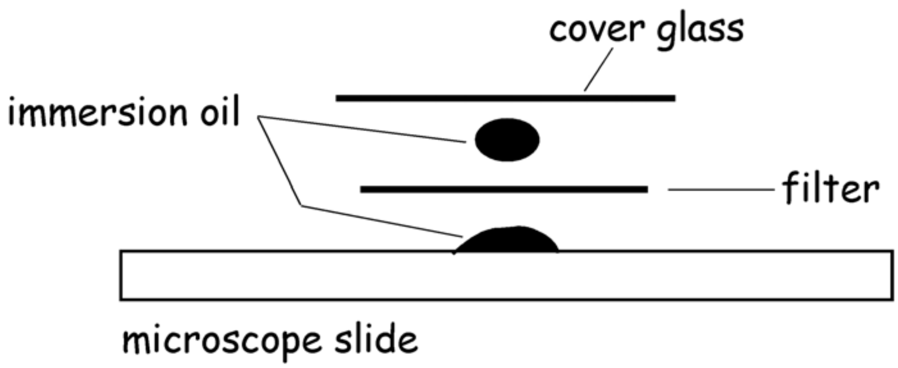

Figure. 3. *Preparation the membrane filter for observation.*

Examination of the filter should be done in a systematic manner and in a way that precludes double counting animals. Sometimes the *T. aceti* will have moved outside of the filtration area before the drying process was completed. Thus, the students will need to examine the area surrounding where the filtration took place to make sure that they count all the animals. However, in a subsequent trial I found that adding 1–2 drops of full strength formalin to fluid to be filtered 1 minute before starting the filter pump killed the *T. aceti* thus preventing the migration.

RESULTS AND DISCUSSION

The results of this exercise for the class in 2002 are shown in (Figure 4). Results for the class in 2003 were similar. As seen by the error bars (±1S.D.), there was considerable variation in the population density estimates among the three replicate samples for the first six weeks of this exercise. Nevertheless, there was a clear region of exponential growth that lasted through week three. Thereafter growth slowed and the population entered a stabilized phase where it appears to have achieved a carry capacity of about 320 *T. aceti* per mL. Because the exercise was stopped after 13 weeks it is not known how long the stationary phase lasts. However, two stock cultures (ca. 500 mL each) in my laboratory have had robust populations for more than two years without the addition of either fresh vinegar or apple. After data collection was stopped the students were given the raw population numbers and were given the following assignment.

1. Plot population size as a function of time twice, once when both axes use an arithmetic scale and again with the population size scale being logarithmic.
2. Using a histogram format, plot the estimated value of *little–r*, from week to week according to the following equation:

   $N_t = N_o e^{rt}$. Solve for *r*: $\ln N_t = \ln N_o + r \cdot t$

   Thus, $r = (\ln N_t - \ln N_o) / t$ (equation 1)

   Here r = the intrinsic rate of natural increase, t = the time that the population has been growing, and N_o starts and N_t ends a period to be calculated.

3. Examine these two figures and discuss the following questions.

A. Did exponential growth occur in this culture? If so where and for how long?

B. Compare the two graphs and discuss which one best illustrated exponential growth.

C. Did the culture reach a carrying capacity? If so, when?

D. In a essay of about 250 words, describe how this exercise demonstrates population growth and the effects of limiting resources on population growth.

E. Develop an abstract that you would use in a manuscript for publication.

F. Offer suggestions to improve this laboratory exercise.

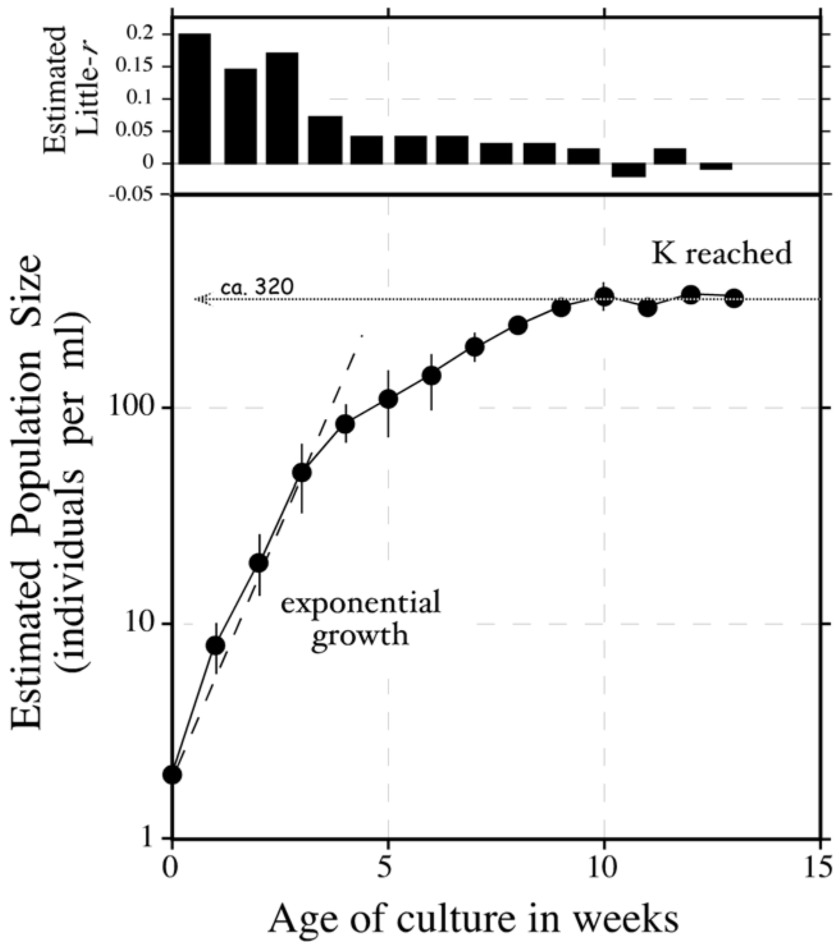

Figure. 4. *Growth of a batch culture of the vinegar eel (*Turbatrix aceti*; Nematoda) over a 13-week period. Upper panel: week-by-week estimates of the value little-r (i.e., 0–1, 1–2, ... 12–13); Lower panel: estimates of the population size as individuals per mL (solid circles). (Lines = error bars as ±1S.D.; error bars are not indicated when they fall within the circle.) (K = carrying capacity of this population.)*

Alternative Enrichment Exercises:

Variations of this exercise including the following: (1) diluting the vinegar to different concentrations; (2) monitoring the pH of the system; (3) monitoring the level of yeast and/or bacteria in the culture; (4) varying incubation temperatures among replicate cultures; (5) comparing cultures incubated in the light vs. the dark; (6) altering the procedures (e.g., buffer the medium, add various natural chemicals or potentially toxic agents).

ACKNOWLEDGMENTS

Two classes of students Biology 247 (*General Ecology*) are acknowledged for their contributions to testing this exercise. I thank W.S. Brooks and an anonymous reviewer who read and improved this manuscript. The anonymous reviewer also suggested the use of formalin.

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Call for Nominations

Honorary Life Award

The **ACUBE Honorary Life Award** is presented to ACUBE members who have made significant contributions and/or service to ACUBE and the advancement of the society's mission. The award is presented at the annual fall meeting of the society.

If you wish to nominate a member of ACUBE for this award, send a Letter of Nomination citing the accomplishments/contributions of the nominee and a *Curriculum Vita* of the nominee to the chair of the Honorary Life Award committee:

Dr. William J. Brett, Department of Life Sciences, Indiana State University, Terre Haute, IN 47809
Voice -- (812) 237-2392, FAX (812) 237-4480, E-mail -- lsbrett@isugw.indstate.edu

Call for Applications – John Carlock Award

This Award was established to encourage biologists in the early stages of their professional careers to become involved with and excited by the profession of biology teaching. To this end, the Award provides partial support for ***upper division undergraduate and graduate students in the field of Biology*** to attend the Fall Meeting of ACUBE.

Guidelines: The applicant must be actively pursuing an ***undergraduate program or graduate work in Biology***. He/she must have the support of an active member of ACUBE. The Award will help defray the cost of attending the Fall meeting of ACUBE. The recipient of the Award will receive a certificate or plaque that will be presented at the annual banquet; and the Executive Secretary will provide the recipient with letters that might be useful in furthering her/his career in teaching. The recipient is expected to submit a brief report on how he/she benefited by attendance at the meeting. This report will be published in Bioscene.

Application: Applications, in the form of a letter, can be submitted anytime during the year. The application letter should include a statement indicating how attendance at the ACUBE meeting will further her/his professional growth and be accompanied by a letter of recommendation from an active member of ACUBE. Send application information or any questions about the Award to:

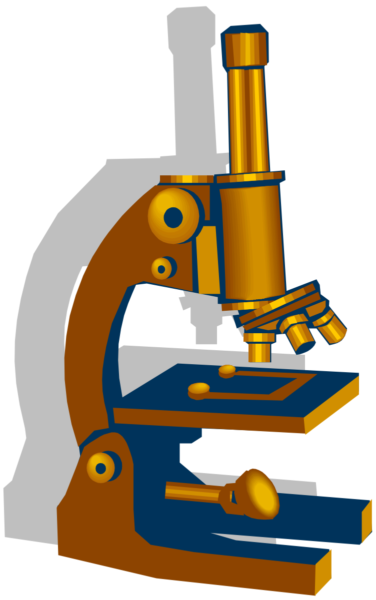
Dr. William J. Brett, Department of Life Sciences, Indiana State University, Terre Haute, IN 47809
Voice:)812) 237-2392; FAX (812) 237-4480; E-mail: lsbrett@isugw.indstate.edu

If you wish to contribute to the John Carlock Award fund, please send your check to:
Dr. Pres Martin, Executive Secretary, ACUBE,
Department of Biology, Hamline University,
1536 Hewitt Ave., St. Paul, MN 55104

Association of College and University Biology Educators 49th Annual Meeting

Southeast Missouri State University
Thursday October 13, 2005-Saturday October 15, 2005

Call for Presentations
Conference Theme: Interdisciplinary Exploration



Interdisciplinary can mean a lot of things. One sort of interdisciplinary exploration was the Lewis & Clark expedition in which many disciplines were used to complete a major project. In addition to scientific sampling, they created maps, described the geological features of the land and waterways, wrote, created images, lived in other cultures, did some politicking and diplomacy, evaded enemies, hunted, built lodging, and used orienteering and survival skills.

Another sort of interdisciplinary exploration is the way in which many biological problems are formulated and studied. For example, modern genomics, which uses statistics, engineering, molecular biology and computer science is an interdisciplinary approach to investigating problems as diverse as systematics and nutrition.

We invite you to submit a paper, poster or workshop on ways you incorporate interdisciplinary exploration or approaches in your biology teaching. Do you team-teach a class with someone from another discipline? Do you give students assignments that are overtly interdisciplinary? Have you designed a course centered on a project or theme that is interdisciplinary (e.g., land use for a particular plot of land, or field courses set in a different environment)? Are your nonmajors science classes becoming a combination of chemistry, physics, biology and earth science? Do your courses contain ethics, economics or global studies?

To submit a proposal for this meeting submit the following form by July 15, 2005 and email it as an attachment to Jill Kruper, Program Chairperson, ACUBE 49th meeting.

Email: jill.kruper@murraystate.edu

Note: At least one presenter for each poster, paper or workshop must be an ACUBE member. (Annual dues are $30.)

Proposed Title:____

Presentation type (Circle one): Poster 45 minute Paper 90 minute workshop

Equipment/Facility needed

______35 mm slide projector
______Overhead projector
______PC computer lab (Sorry, no MAC equipment available)
______PC projection system
______Other (Please explain)

Name of Presenter______________________________________

Professional Address____________________________________

Phone:________________ email__________________________

Name of co-Presenter ____________________________________

Professional Address ____________________________________

Phone ________________ email __________________________

Abstract (200-250 words):

The 49th annual meeting of **ACUBE** will be held at Southeast Missouri State University in Cape Girardeau, MO. Join us for "Interdisciplinary Exploration."

Cape Girardeau is a bustling city of 37,000 people nestled on the banks of the Mississippi. Beginning as a trading post in the late 1700's, Cape Girardeau was an early stop on the Lewis and Clark expedition, was the site of a battle in the Civil War, and today retains much of its character and charm in the riverfront business district, a Missouri Main Street community known as "Old Town Cape." As a regional center (and the largest city between St. Louis and Memphis), Cape Girardeau offers excellent restaurants, shopping, and a wealth of antiquing possibilities. Known for its medical, educational and retail resources, over 90,000 people come to work daily in Cape.

Southeast Missouri State University sits on a hill above the Mississippi about 1 mile from Old Town Cape. Originally a teacher's college, Southeast today is a comprehensive regional university offering bachelor's and master's degrees to its 8500 students. Our meetings will be held in the University Center meeting rooms, with some sessions in the adjacent Kent Library. All meeting rooms are handicapped accessible. The campus is on rolling terrain, nice for a lunch time walk. See images at www.semo.edu

Nearby state parks in Missouri and Illinois include the Trail of Tears with its rugged terrain for hiking and Big Oak Tree State Park near New Madrid MO with its remnant swamp ecology in MO. Giant City State Park and Little Grand Canyon (Fern Cliff) are state parks in Illinois with interesting geological formations and plants. Horseshoe Lake recreation area near Olive Branch IL offers fishing and birding.

The Cape Girardeau Convention and Visitors Bureau website provides much information on activities and attractions in the Cape Girardeau region. Go to http://www.capegirardeaucvb.org/

Cape Girardeau's weather in October is likely to be warm, but visitors are advised to check the forecast before traveling (it has snowed in October recently). Dress for most all activities in Cape is casual and comfortable.

For more information on proposing a presentation or registering for this meeting, or for housing info and driving directions go to http://acube.org/ and click on the meetings button.

ACUBE 2005 Annual Meeting Registration Form

Southeast Missouri State University, Cape Girardeau, MO

October 13-15, 2005

NAME: ________________________________ DATE: ______________

TITLE: ____

DEPARTMENT:____

INSTITUTION: ____

STREET ADDRESS: ____

CITY: __________________ STATE: _______ ZIP CODE: _______

ADDRESS PREFERRED FOR MAILINGS: __________________________

____

CITY: __________________ STATE: _______ ZIP CODE: _______

WORK PHONE: ___________ FAX NUMBER: ____________________

HOME PHONE: ___________ EMAIL ADDRESS: _________________

Registration Fee: Includes meals Friday-Sat noon, refreshments at breaks, and field trips.

Membership status	By 9/20/05	After 9/20/05
Regular Member	$ 85	$100
Regular member + 2006 dues	$115	$130
New Member (includes 2006 dues)	$115	$130
Non-Member	$115	$130
Non-Participating guest/spouse	$ 55	$ 55
Student (Grad or Undergrad)	$ 55	$ 55
K-12 teacher	$ 55	$ 55
Friday evening dinner only	$ 15	$ 15

TOTAL ENCLOSED (Please make checks payable to ACUBE) ___________

Sorry, checks or money orders only.

Field Trips: Indicate the trip(s) you plan to attend. Space is limited, register early!

_______ Thursday afternoon: 1 pm- 5 pm: Little Grand Canyon geology and biology trip

_______ Friday morning: 7:00 - 10:00: Birding at Horseshoe Lake, IL

_______ Friday afternoon: 3:00 - 5:30: The Red House (Lewis and Clark interpretive center) & searching for references to biology in material goods (antiquing)

Special needs (food, facilities, etc.):

Please send registration form and payment to:

Dr. Margaret Waterman
ACUBE Local Arrangements Chair
Department of Biology, MS 6200
Southeast Missouri State University
Cape Girardeau, MO 63701

mwaterman@semo.edu

(573) 651-2381

ACUBE Annual Meeting Housing Information.

Recommended Area Hotels/Motels. Watch for ACUBE discount coming soon.

All of the hotels below are off exit I-55 #96, near the mall and shopping and two miles from campus.

Drury Lodge
104 South Vantage Drive
573-334-7151
573-334-7151 (Fax)
AAA Rating: 2 Diamonds

Drury Suites
3303 Campster Drive
573-339-9500
800-DRURYINN (Toll Free)
AAA Rating: 3 Diamonds

Hampton Inn
103 Cape West Parkway
573-651-3000
573-651-0882
AAA Rating: 3 Diamonds

Pear Tree Inn
3248 William St.
573-334-3000
573-334-3000 (Fax)
AAA Rating: 2 Diamonds

Victorian Inn
3265 William Street
573-651-4486
573-651-3970 (Fax)
800-331-0445 (Toll Free)
AAA Rating: 3 Diamonds

Holiday Inn Express
Next to Victorian Inn
Coming Spring 2005

Off I-55 exit 99, near county park

Super 8 Motel
2011 North Kingshighway
573-339-0808
573-339-2060 (Fax)
AAA Rating: N/R

Bed & Breakfast Inns

Bellevue Bed and Breakfast
312 Bellevue
Cape Girardeau, MO 63701
800-768-6822 or 335-3302
www.bellevue-bb.com/

Near University (1 mile) and downtown (3 blocks)

Rose Bed Inn Bed & Breakfast
611 South Sprigg
Cape Girardeau, MO 63703
332-ROSE (7673)
866-ROSEBED (767-3233)
www.rosebedinn.com

2 miles south of University

Neumeyer's Bed and Breakfast
25 S. Lorimier St.
Cape Girardeau, MO 63703
335-0449 or 888-423-5184
www.capegirardeaucvb.org/neumeyers.html

Downtown, 1.5 miles from university

Campgrounds

Cape Camping and RV Park
(Now Open)
(573) 332-8888 or (800) 335-1178
1900 North Kingshighway
Cape Girardeau, MO 63701
- Full hook-ups with cable tv and wireless internet (107 total camping sites)
- Picnic tables and fire rings for grilling at every site
- Swimming pool with bathhouse
- www.capervpark.com

Suburban location, good for RV.

Trail of Tears State Park
573-334-1711
Highway 177
Jackson, MO 63755
Has full hook-up sites; basic tent sites; and electric only sites.

Note: this is lovely!! About 12 miles from University.

ACUBE

Association of College and University Biology Educators

NAME: ______________________________ DATE: ______________

TITLE: ____

DEPARTMENT: ____

INSTITUTION: ____

STREET ADDRESS: ____

CITY: ____________________ STATE: __________ ZIP CODE: __________

ADDRESS PREFERRED FOR MAILING: ______________________________

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CITY: ____________________ STATE: __________ ZIP CODE: __________

WORK PHONE: ______________ FAX NUMBER: ____________________

HOME PHONE: ______________ EMAIL ADDRESS: ____________________

MAJOR INTERESTS	**SUB DISCIPLINES:** (Mark as many as apply)	
( ) 1. Biology	( ) A. Ecology	( ) H. Molecular
( ) 2. Botany	( ) B. Evolution	( ) I. Developmental
( ) 3. Zoology	( ) C. Physiology	( ) J. Cellular
( ) 4. Microbiology	( ) D. Anatomy	( ) K. Genetics
( ) 5. Pre-professional	( ) E. History	( ) L. Ethology
( ) 6. Teacher Education	( ) F. Philosophy	( ) M. Neuroscience
( ) 7. Other __________	( ) G. Systematics	( ) N. Other __________

RESOURCE AREAS (Areas of teaching and training): ____________________

____

RESEARCH AREAS: ____

____

How did you find out about ACUBE? ______________________________

Have you been a member before: __________ If so, when? ____________________

DUES (Jan-Dec 2006) Regular Membership $30 Student Membership $15 Retired Membership $5

Return to: Association of College and University Biology Educators, Attn: Pres Martin, Executive Secretary, Department of Biology, Hamline University, 1536 Hewitt Avenue, Saint Paul, MN 55104