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Each year as the Microbiology Education Editorial Board meets to discuss the submitted papers, we marvel at the creative ideas that are presented in the manuscripts. This year we noticed multiple manuscripts designed to reach a younger student population and novel mechanisms to achieve this goal. In the article by Abrahamsen, service learning is presented as a mechanism to enhance undergraduate education while taking the discipline to a younger audience. Abrahamsen relates her experiences partnering her students at Bates College with middle and high school students, creating a mutually beneficial learning experience for both the undergraduate and precollege students. Additionally, the paper by Miller et al. discusses the use of the Internet in teaching middle school microbiology. While this paper does not directly discuss service learning, one could certainly envision undergraduates pairing with middle school students to discuss the microbial adventures the students encountered in the program. Service learning is taking on a greater sense of importance in the education of undergraduates at various institutions, and I am pleased that Microbiology Education is playing a role in this transformation by providing mechanisms to enhance this type of education.

As with all aspects of life, new technology (e.g., the web and state-of-the-art equipment) is providing many opportunities for educators. In addition to the paper by Miller et al., the papers by Takayama and Strong relate educational strategies that utilize the web and web-based programs to teach microbiology. Since we, as educators, are always looking for good examples of how to best utilize this important and ever-growing resource in our teaching, I am happy that Microbiology Education is able to provide some quality activities. Along with the web, state of the art equipment, such as the flow cytometer, is finding its way into the undergraduate classroom. The paper by Booth et al. demonstrates how to use flow cytometry for educational purposes.

Enjoy this fifth volume. May it help you to view your teaching and learning in ways not previously considered and stimulate you to consider your own research in microbiology education.

Respectfully,

Jeffrey J. Byrd
Chair, Editorial Board for Microbiology Education
Three-Dimensional Visualizations in Teaching Genomics and Bioinformatics: Mutations in HIV Envelope Proteins and Their Consequences for Vaccine Design

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This project addresses the need to provide a visual context to teach the practical applications of genome sequencing and bioinformatics. Present-day research relies on indirect visualization techniques (e.g., fluorescence-labeling of DNA in sequencing reactions) and sophisticated computer analysis. Such methods are impractical and prohibitively expensive for laboratory classes. More importantly, there is a need for curriculum resources that visually demonstrate the application of genome sequence information rather than the DNA sequencing methodology itself. This project is a computer-based laboratory lesson that enables students to analyze information that may be perceived as relatively challenging and put it into a practical context. Its versatility makes it adaptable to most courses in the biological sciences. A case study examining mutations in HIV-1 genome sequences obtained from a cohort of HIV-seropositive patients was presented to 54 third-year virology students. Students applied information obtained from bioinformatics to analyze the high genomic sequence mutation rates of HIV-1 with respect to epidemiology, molecular structure, and therapeutic design strategy. Based on these analyses, students developed three-dimensional models of the viral protein structures that allowed visual mapping of sequence evolution. This approach facilitated students’ appreciation for the application of genomic sequence information into a practical context.

Students collaborated to identify a region of the genome that mutated less frequently and would therefore be a target candidate for vaccine development. This active problem-solving approach enabled students to gain an appreciation for the challenge of developing vaccines or therapeutic drugs for a virus such as HIV-1, which mutates so frequently. The lesson was developed to facilitate critical thinking through the application of one type of information (DNA or protein sequence) into three-dimensional models.

One of the key criteria for an authentic learning experience is fidelity of context (11, 22, 28, 34). The goal of this project was to engage students in an open-ended learning experience based in a relevant context. In addition to strengthening students’ general inquiry-based research skills, the presentation of areas such as bioinformatics and genomics as a case study allows students to reflect on the particular relevance of highly analytical subjects.
The total time devoted to the lesson was two 3-hour computer laboratory sessions, preceded by a 1-hour tutorial. The key concepts upon which the lesson is based are:

- HIV-1 exhibits a high mutation rate during genome replication due to the low fidelity of the virus-specific enzyme, reverse transcriptase.
- A population of HIV-1-infected individuals may display wide genetic heterogeneity of viral subtype nucleotide sequences; these patterns can change over time.
- Bioinformatics enables analysis of specific patterns of changes in genomic sequences that can reveal crucial information about the molecular biology and epidemiology of HIV-1.
- The changes in viral gene sequence may significantly affect the structure of viral proteins and hence present challenging implications with regard to targets for vaccine and/or therapeutic design.

The lesson is based on a published study (19) in which the pattern of HIV-1 evolution was compared to CD4+ T cell decline in 15 subjects. In the study, the subjects were followed from seroconversion for up to 4 years (at 6-month intervals). Mutations of clonal variants of HIV-1 (clade B) were analyzed via PCR amplification of a 285 bp fragment from the hypervariable V3 loop region of the env gene, encoding the viral glycoprotein gp120.

The background information was presented to the students as a case study scenario:

**Background, HIV-1 evolution.** HIV-1, the causative agent of AIDS, exhibits high mutation and replication rates that facilitate its adaptation to changes in the host environment as well as its eventual resistance to certain antiretroviral therapies. Like other retroviruses, HIV-1 has a much higher mutation rate than is typically found in organisms whose genomic replication does not involve reverse transcription. The in vivo forward mutation rate in HIV-1 has been estimated to be $3.4 \times 10^{-5}$ mutations/base pair/replication cycle (5, 18, 20, 27).

HIV-1 infected individuals may display wide genetic heterogeneity (diversity) of viral subtype nucleotide sequences. The host environment can affect the genetic composition of a given virus pool. For example, instability could be generated by a dynamic host immune response or by differential display of coreceptors. If the destabilising force selected randomly against the broad range of existing variants, diversity would most likely be reduced to those variants that were initially most numerous. On the other hand, if selective forces such as the immune response targeted primarily those variants that were most abundant (frequency-dependent selection), overall viral load would be reduced but genetic diversity would be maintained, since the less frequent viral strains would still be present.

By examining patterns of diversity during HIV-1 evolution, we can observe the type and efficiency of selection forces influencing viral evolution, as well as how the virus adapts to those forces. Furthermore, the analysis of frequency and diversity of viral mutation provides valuable information for the framework of potential drug therapy-vaccine development for a given population.

**Case study.** A study has been conducted on the relationships between the pattern of HIV-1 evolution in 15 seroconverting patients and the rate of CD4+ T cell decline. CD4+ T cell depletion is a characteristic of HIV-1 infection, as the virus infects CD4+ T cells. The changes were monitored at frequent intervals over a period of up to 4 years. The individuals selected for the study were followed from the point of HIV-1 seroconversion and had attained different levels of CD4+ T cells.

Genetic sequence variation was analyzed for a 285 base pair region around the third hypervariable (V3) domain of the viral env gene. The env gene product, membrane protein gp120, binds to the CD4 receptor site on T lymphocytes and is involved with viral entry into the cell. This region was therefore chosen for analysis because it is an important site of host-virus interaction and is known to tolerate frequent mutations. Blood samples were collected from the 15 subjects at 6-month intervals for up to 4 years and analyzed for virologic and immunologic studies. PCR was used to amplify the 285 bp region from peripheral blood mononuclear cells. The PCR-amplified DNA fragments were cloned into a plasmid and sequenced. The results for the variant clones are tabulated on the following pages.

The students, working in pairs, were assigned one subject for their case study. Each pair of students was presented with a tabulated summary of data tracking all subjects’ disease progression (represented as CD4+ T cell counts) and number of HIV-1 clones detected in each subject at specific visits (sample of data shown in Table 1). Each pair of students also received the Genbank accession numbers for all of the HIV-1 env sequences from each visit for their subject (available at http://www.microbelibrary.org/Journal/TakayamaGenbankAccession.pdf).

The students are then presented with the following challenge:

**Research project.** You are working with molecular virologists, clinical virologists, and physicians to develop an improved drug therapy regimen for HIV-1 patients. The env sequences analyzed in this study encode a protein that binds to a receptor on the T-cell surface, allowing the virus to enter and ultimately destroy the cell (and consequently the body’s immune capabilities). Developing drugs that block this binding have had limited short-term success, the major impediment being the rapid mutation rate of the env gene, which renders highly specific blockers ineffective. You will examine patterns in the mutations occurring in these env sequences over time.

Each pair of students initially discussed the data for all subjects to examine and critique the available information with regard to potential patterns of correlations. The students then performed genomic sequence alignment analyses (for the 285 bp V3 region) of all viral clones for their subject at the nucleotide (Fig. 1) and protein sequence levels (Fig. 2). The sequences are available on the Genbank public database, and students employed the open source web-based
TABLE 1. Summary of data on subjects analysed for HIV-1 mutation and CD4⁺ cell count*

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program Biology Workbench (http://workbench.sdsc.edu/) to perform their analyses.

Students engaged in collaborative discussions to analyze their sequence alignments for their subjects using the following questions as a guide:

**Questions.**

1. Upon analysis of nucleotide mutations over time for each of the clones in your subject, are there specific nucleotide positions that mutate more frequently than others?

2. Do they mutate in predictable ways (have the same change) over time?

3. How do the mutation patterns of the protein sequences compare to those of the nucleotide sequences?

4. Based on your analyses, which viral clones are most closely related evolutionarily? Which clones are least related evolutionarily?

5. Discuss within your laboratory group and then as a whole class: if you were to design a drug or vaccine to target a specific portion(s) of the HIV-1 env glycoprotein, which region would you target based on your sequence analyses? Why?

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* Some of the visit numbers are not sequential. In all cases visit 1 represents the first time the subject was evaluated. The subsequent time points represent six-month intervals from the initial visit. Thus, if a subject missed their six-month appointment their visits would be numbered 1, 3, 4, etc. (Reference: 19).
based program, Protein Explorer (21), was utilized to model protein sequence alignments and test the students’ predictions. Protein Explorer is a powerful program that enables three-dimensional (3-D) modeling and visualization of protein structures. Multiple sequence alignments were mapped using Protein Explorer and ConSurf (8) to identify amino acid residue positions that remained highly conserved in comparison to those that mutated significantly (Fig. 3). Students compared protein sequence alignments of their subjects’ gp120 sequences against the sequence of one of several gp120 protein structures available in the Protein Data Bank (PDB) (1) (e.g., PDB accession number 1G9M (15, 16)). The interactivity of Protein Explorer allows students to rotate the 3-D structure of their gp120 alignment, zoom in and out, and highlight specific polypeptide chains as well as specific amino acid residues. Furthermore, the PDB structures of the gp120 chain are represented together with the CD4 receptor and an epitope-specific antibody, enabling students to examine potential steric interactions and interceptions between specific exposed regions of gp120.

FIG. 1. Biology Workbench nucleotide sequence alignment demonstrating subject 1 HIV-1 clone diversity at visit number 1. The alignment was obtained using the boxshade function in Biology Workbench.
FIG. 2. Biology Workbench protein sequence alignment demonstrating subject 1 HIV-1 clone diversity at visit number 1. The alignment was obtained using the boxshade function in Biology Workbench.
Students were able to examine the patterns of HIV-1 evolution in patients by analyzing the nucleotide sequences from a region of the *env* gene. They made predictions about which regions of the corresponding amino acid sequence may serve as potential targets for drug or vaccine design strategy. However, comparative sequence analysis on its own cannot provide structural information that is critical for scientists involved in pharmaceutical design. Furthermore, for the student to fully appreciate the consequences of the high mutational frequencies of HIV-1, the visualization of these events in three dimensions provides an invaluable comparative resource. Students were able to appreciate the functional consequences of mutations in specific regions of the HIV-1 genome by visualizing the structural effects in 3-D representations of gp120. Indeed, other educators have also reported that molecular visualizations are effective in facilitating student comprehension of protein structure and function.

Learning outcomes were determined by defining specific learning objectives for the lesson and analyzing students’ responses to conceptual questions designed to assess achievement of those objectives. Performance assessments judge student abilities to use specific knowledge and research skills to solve a problem or make an analysis. Student comprehension and problem-solving ability was assessed using the performance assessment questions. Questions were graded by the instructor. A summary is presented in Table 2.

Students were asked to answer these questions before and after the computer laboratory sessions. The questions were designed to assess different levels of cognitive ability, focusing on the central theme of the application of genomics. Student responses were scored on a scale of 1 to 4, using the following rubric for classifying comprehension: 1, little or no understanding of the topic, failed to meet objective; 2, some understanding of topic, answer had some relevance towards meeting objective; 3, sufficient understanding of topic, answer is generally correct but lacks strategic or innovative detail; 4, outstanding understanding of topic, answer indicative of further reflection and application (transfer) of concepts. The outcomes are presented in Table 3.

For each objective, student performance improved markedly following the computer tutorial. The outcomes indicate that student comprehension of the application of bioinformatics is enhanced by this investigative approach. Furthermore, because the objectives were presented within a case study, emphasizing relevance and context, some answers extended beyond the expectations of the instructor. For example, for question D, a student replied, “In order to

---

**FIG 3.** 3-D structure summarizing data from multiple sequence alignment of subject 1, visit 1 HIV-1 protein sequences modeled on HIV-1 gp120 core complexed with the CD4 receptor and a neutralizing human antibody. Magenta (spacefill) residues represent highly conserved regions; blue (spacefill) residues represent variable regions of gp120. The CD4 receptor, antibody light chain, and antibody heavy chain are indicated.
TABLE 2. Student performance objectives and assessment questions.

<table>
<thead>
<tr>
<th>Objective</th>
<th>Conceptual Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. To understand the biological relevance of the env gene</td>
<td>A. One primary target in HIV-1 that comes to mind when developing a drug or vaccine is the env gene, which encodes gp120. Why?</td>
</tr>
<tr>
<td>B. To understand how bioinformatics is utilized for genomic analysis.</td>
<td>B. What, in your understanding, does bioinformatics reveal to us about a genomic sequence of interest?</td>
</tr>
<tr>
<td>C. To apply conceptual knowledge towards developing a computer-based method to solve a bioinformatics problem.</td>
<td>C. If you were using bioinformatics to determine the most appropriate target for vaccine development against a region of gp120, draw a flowchart depicting your approach. Assume you have all the necessary bioinformatics analysis software and databases at your disposal.</td>
</tr>
<tr>
<td>D. To understand that comparative analyses of gene and protein sequences alone are insufficient for providing comprehensive biological functional information; i.e., structural information is also needed.</td>
<td>D. One of your colleagues has performed sequence alignments of the env gene sequence, and the gp120 protein sequence. She has decided on a vaccine target region based on this information. Please provide your critique on this.</td>
</tr>
</tbody>
</table>

TABLE 3. Student outcomes of performance assessment

<table>
<thead>
<tr>
<th>Objective</th>
<th>Level of student cognitive ability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Objective A</td>
<td></td>
</tr>
<tr>
<td>Scores before computer session</td>
<td>13</td>
</tr>
<tr>
<td>Scores upon completion of computer session</td>
<td>0</td>
</tr>
<tr>
<td>Level of significance (χ² test): P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Objective B</td>
<td></td>
</tr>
<tr>
<td>Scores before computer session</td>
<td>44</td>
</tr>
<tr>
<td>Scores upon completion of computer session</td>
<td>0</td>
</tr>
<tr>
<td>Level of significance (χ² test): P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Objective C</td>
<td></td>
</tr>
<tr>
<td>Scores before computer session</td>
<td>50</td>
</tr>
<tr>
<td>Scores upon completion of computer session</td>
<td>0</td>
</tr>
<tr>
<td>Level of significance (χ² test): P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Objective D</td>
<td></td>
</tr>
<tr>
<td>Scores before computer session</td>
<td>50</td>
</tr>
<tr>
<td>Scores upon completion of computer session</td>
<td>10</td>
</tr>
<tr>
<td>Level of significance (χ² test): P &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

¹Student responses were scored on a scale of 1 – 4 (refer to text for rubric definitions); values indicate the number of students whose responses to a specific objective question corresponded to that cognitive category.
²A total of 53 students completed the assessment questions before the computer session.
³A total of 51 students completed the assessment questions after the computer session.
ensure that the potential vaccine target region is present on an exposed surface where it can be recognized by the immune system, protein modeling software may be used to predict the protein structure. Further in vitro and in vivo testing must be conducted to ensure that the target region can be expressed in a functional folded form that closely resembles the gp120 epitope and the target region is immunogenic.

The value of 3-D computer modeling to examine the consequences of the gp120 mutations was appreciated by students. The students were able to explore the structure and crucial regions involved in receptor binding and recognition. Furthermore, the collaborative framework of the learning experience enhanced the students’ problem-solving strategies. Other studies have also demonstrated the successful outcomes of collaborative and problem-based learning mediated by technology (13, 24, 25, 26). The computer project described here can certainly be used in conjunction with comprehensive supplementary introductions to the areas of bioinformatics and functional genomics (such as those described in 2, 14) for further in-depth exploration of specific protein families or evolutionary and functional relationships among conserved proteins.

ACKNOWLEDGMENTS

I wish to acknowledge the support of the University of New South Wales Innovative Teaching and Educational Technology Fellowship program, which allowed me to develop this project. The idea for this lesson plan evolved from a tutorial, “Exploring HIV Evolution,” presented in June 2001 by Sam Donavan at the Bioquest workshop, “Microbes Count: Problem Posing, Problem Solving, and Persuading Peers in Microbiology Education.” I am grateful to Bioquest for the opportunity to participate in the workshop and to Eric Martz, University of Massachusetts—Amherst, for introducing me to Protein Explorer.

REFERENCES

ASM’s Education Board sponsors a variety of fellowship programs for postdoctoral scientists, graduate and undergraduate students. Below is a listing of the fellowship programs available.

**ASM/NCID Postdoctoral Research Associates Program**
Encourages postdoctoral scientists to conduct research in infectious diseases, medical microbiology, and immunology at the National Center for Infectious Diseases. The fellowship is for two years. **Deadline for application:** November 15.

**Robert D. Watkins Minority Graduate Fellowship**
Encourages minority graduate students to conduct research in the microbiological sciences. The fellowship is for three years. **Deadline for application:** May 1.

**ASM Undergraduate Research Fellowship**
Encourages undergraduate students to conduct a research project in the laboratories of ASM sponsoring members at their home institution for a minimum of eight weeks and present research findings at the ASM General Meeting. **Deadline for application:** February 1.

**ASM Minority Undergraduate Research Fellowship**
Encourages minority undergraduate students to conduct a research project in the laboratories of ASM sponsoring members for a minimum of eight weeks and present research findings at the ASM General Meeting. **Deadline for application:** February 1.

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Tel: (202) 942-9283 or (202) 942-9295
Fax: (202) 942-9329
Efficacy of MedMyst: an Internet Teaching Tool for Middle School Microbiology

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Rice University, Houston, Texas, 77005

Can web-based technology be used to effectively introduce or reinforce aspects of microbiology to middle school students? This central hypothesis examines whether brief exposure to a web adventure format containing virtual lab experiments and computer games within an engaging story line can impact student learning. An episodic adventure series, MedMyst (http://medmyst.rice.edu), focuses on infectious diseases and the microbes that cause them. The website is not intended to replace classroom instruction, but rather to engage students in problem-solving activities not likely to be encountered elsewhere. It also provides scientists with a resource to introduce microbiology to adolescent audiences through outreach activities. In the online adventure, the player (student) enters a futuristic world in which he or she becomes a “Reconstructor,” a member of an elite team charged with preventing the spread of infectious disease. The series consists of three “missions,” each lasting approximately 30 to 40 minutes and designed to address a limited set of learning objectives. Middle school students participated in the creation of the characters and the stylized design through focus groups. Classroom teachers oversaw the alignment of the web adventure objectives with the National Science Content Standards. Scientists and clinicians reviewed the web adventure for content and accuracy. A field test involving over 700 students from nine different schools assessed the knowledge gains attributable to playing MedMyst. Gain scores from pretest to posttest indicated that middle school students retained important information by interacting with the online material for as little as 30 minutes per adventure; however, gains for high school students were less persuasive, perhaps indicating a different learning tool or content is required for this age audience.

The roadmap for incorporating best practice in education with the creativity of multimedia is based upon the type of learning environment described in Benchmarks for Scientific Literacy (1) and the National Science Education Standards (6, 20). The alignment of the MedMyst content with these standards was central to the materials development process. A summary of the specific standards incorporated is provided in Table 1.

Much of what is available on the web is a compendium of information with little structure or few scaffolds on which students might build their own knowledge. Some observers have charged that this has led to “lazy” learning models, “where the student is simply confronted with a vast resource and left unguided” (23). This type of exploratory learning can sometimes have an effect opposite from that which was intended. It can cause disorientation, difficulty in navigating from point to point, and cognitive overload (24). To overcome these challenges, several researchers have used a storyline or narrative as a means of providing structure to multimedia materials (12, 13). Researchers have proposed “that narrative shapes our knowledge and experience and is central to the processes of teaching and learning because it aids reconstruction, retrospection, prediction and memory as well as motivation” (21). The use of narrative in combination with multimedia has several advantages:

- It sets a context or situates a problem that is to be solved (4, 7, 8).
- It grounds the use of text, graphics, animation, voice, music, and interactivity with a certain mood or theme (16).
- It allows the layering of learning objectives so that an array of objectives as described by Bloom’s Taxonomy (3) can be woven into the story or problem.
- It exploits theories of constructivism that suggest narrative is a powerful instructional tool (5, 25).

The survey, “Internet Access in U.S. Public Schools, Fall 2001,” revealed that 99% of public schools in the United States had Internet access (19). Recent national studies indicated that 70% of American households with children ages 2 to 17 years have computers (10) and 52% are connected to the Internet (2, 22). Our own 1999 survey of middle school students in the Houston metropolitan area indicated that 80% report having Internet access at home (14). Therefore, crafting materials for this new mode of delivery is forward looking, more economical than print media, and has tremendous dissemination advantages. Creative electronic resources can reach students not only in the formal classroom environment, but can also become a part of the education environment in homes or museums and among the home-schooled population.

Students of both genders reported their dominant form of computer use was “playing games” (11, 17). These preferences speak to the viability of using innovative “game playing” as a way to teach adolescents science. If gaming strategies and animations can be designed for microbiology content, might this lead to student learning?

MATERIALS

Development of web adventures. Medical Mysteries (MedMyst) was produced through an interactive design process that engaged researchers, clinicians, educators, and students. This process of moving from Science Standards to

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The final production of the interactive web adventures is described elsewhere (15). Three of the four authors of this study were also involved in the website design. Prior to creating the web adventure, an informal survey of teachers and scientists was conducted to determine what middle school students should know about microbiology and infectious diseases. The distillation of survey responses, in combination with the Benchmarks (1) and Science Education Content Standards (20), led to the specification of learning objectives. These identified objectives were translated into learning segments within a narrative, then outlined in a screen-by-screen storyboard. Macromedia FLASH was selected as the programming tool because of its web capabilities. Animation and sound were added to the storyline only where it supported the content without unreasonably enlarging the file size. Aware that some users may not always have a high-speed connection to the Internet or a machine with a fast processor, the creators weighed each programming decision.

<table>
<thead>
<tr>
<th>Instructional objectives covered in MedMyst</th>
<th>National science content standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Apply the scientific method to the investigation of a mystery disease</td>
<td><strong>Science as inquiry</strong></td>
</tr>
<tr>
<td>• Distinguish between essential and nonessential information in determining the cause of the mystery disease</td>
<td>Content standard A: all students should:</td>
</tr>
<tr>
<td></td>
<td>Develop abilities necessary to do scientific inquiry</td>
</tr>
<tr>
<td></td>
<td>Understand about scientific inquiry</td>
</tr>
<tr>
<td></td>
<td><strong>Life science</strong></td>
</tr>
<tr>
<td>• Understand the structure of the different infectious agents: bacteria, fungi, helminthes, prions, protozoa, and viruses</td>
<td>Content standard C: all students should develop an understanding of:</td>
</tr>
<tr>
<td></td>
<td>Structure and function in living systems</td>
</tr>
<tr>
<td></td>
<td>Reproduction and heredity</td>
</tr>
<tr>
<td></td>
<td>Regulation and behavior</td>
</tr>
<tr>
<td></td>
<td>Diversity and adaptations of organisms</td>
</tr>
<tr>
<td>• Summarize the process of bacterial and viral reproduction</td>
<td><strong>Science and technology</strong></td>
</tr>
<tr>
<td>• Examine the role of the immune system in fighting infectious diseases</td>
<td>Content standard E: all students should develop:</td>
</tr>
<tr>
<td></td>
<td>Abilities of technological design</td>
</tr>
<tr>
<td></td>
<td>Understandings about science and technology</td>
</tr>
<tr>
<td></td>
<td><strong>Science in personal and social perspectives</strong></td>
</tr>
<tr>
<td>• Explain what a vaccine is, how it is made, and how it prevents disease</td>
<td>Content standard F: all students should develop understandings of:</td>
</tr>
<tr>
<td>• Formulate an effective method for distributing oral rehydration solution</td>
<td>Personal health</td>
</tr>
<tr>
<td></td>
<td>Populations, resources, and environment</td>
</tr>
<tr>
<td></td>
<td>Natural hazards</td>
</tr>
<tr>
<td></td>
<td>Risks and benefits</td>
</tr>
<tr>
<td></td>
<td>Science and technology in society</td>
</tr>
<tr>
<td></td>
<td><strong>History and nature of science</strong></td>
</tr>
<tr>
<td>• Understand the pathology and treatment of various infectious diseases, especially smallpox and cholera</td>
<td>Content Standard G: all students should develop understandings of:</td>
</tr>
<tr>
<td>• Discover how natural disasters and human activity can adversely affect the environment</td>
<td>Science as a human endeavor</td>
</tr>
<tr>
<td>• Correlate the appearance of clean water with disease</td>
<td>Nature of science</td>
</tr>
<tr>
<td>• Simulate the spread of an infectious disease</td>
<td>History of science</td>
</tr>
</tbody>
</table>
in order to make the site usable on slower machines.

The storyline allows the introduction of a variety of mechanisms to present and test learning outcomes as described by Bloom’s Taxonomy (3). Objectives range from the simplest Knowledge level in which the student is asked for factual answers, testing recall and recognition, to the more complex levels of Application, Analysis, or Synthesis, which require students to predict what would happen, draw conclusions based upon new information, or propose an alternative. For example, in order to enter the lab, the player must know the answer to a content question; in another scene, the player must draw conclusions from an experiment’s results before being allowed to proceed.

By way of illustration, the objectives from Mission One are:

1. Simulate an experiment demonstrating Koch’s Postulates.
2. Apply the steps of Koch’s Postulates to the identification of a disease in humans.
3. Identify the six types of infectious agents and their characteristics.
4. Describe the role of the immune system in fighting diseases.
5. Associate modes of transmission with each type of pathogen.
6. Distinguish the different treatments and preventative measures that are most effective for each pathogen.
7. Match types of infectious agents to the specific disease they cause.
8. Recognize persons from history who made contributions to Germ Theory.

The storyline is the thread that pulls the students through the content and levels of questioning in a meaningful way. In MedMyst the underlying premise is that the player (student) enters a futuristic world in which he or she assumes the role of a “Reconstructor” charged with preventing the spread of infectious diseases. Figure 1 presents the initial context that the player encounters.

At the beginning of each adventure, the student is presented with a “problem” that must be solved. During a mission, students conduct field and laboratory investigations with the aid of the MedMyst characters. Each mission can be played within one class period (approximately 40 to 50 minutes). It is recommended that Mission One be played first because it covers the basics, and as the title indicates, it serves as an “orientation” to the concepts and the characters; however, either Mission Two (cholera) or Mission Three (smallpox) may be chosen to follow Mission One. Because each is a self-contained problem, either one may be played without reliance on the other Mission. Cholera and smallpox were selected because they represent two different categories of infectious agents (bacteria and viruses). The series unfolds along these lines:

Mission One: Orientation in Orb. The mission begins in the year 2254 in the Neuropolis Center for Disease Control. Prior to assignment to the field as new members of the Reconstructors, students must successfully complete five challenges based on their knowledge of microbes and infectious disease. The games require students to demonstrate their knowledge of Germ Theory, infectious agents, infectious disease vectors, the immune system, and treatment and preventative measures for infectious diseases.

Mission Two: Peril in Prokaryon. In the Neuropolis outpost of Prokaryon, the student must stop an outbreak of an infectious disease that threatens a refugee camp. The player must conduct an epidemiological study; identify the infectious agent through a microscope; discover the source of the illness through interviews, maps, and a case control study; and determine how to treat and prevent the illness from spreading.

Mission Three: Nemesis in Neuropolis. Working against time, each player must solve the mystery of an infectious disease never seen before in Neuropolis. Is it the result of bioterrorism? How can it be stopped? To succeed, players must piece together clues from the life of the first victim and his family, use a virtual electron microscope to identify the infectious agent, and decide whether to implement a vaccination program for the community.

Throughout the adventure, interactive puzzles and quizzes are used to ensure that the students have understood the information. Figures 2 and 3 provide specific illustrations of the interactivity. If a student has difficulty with the knowledge assessments, hints are provided, usually by one of the characters in his/her role as tutor (Fig. 4).

METHODS

Subjects. In order to secure a student population for testing, teachers from around the United States were recruited at MedMyst workshops presented at the regional and national meetings of the National Science Teachers Association. As an incentive, a stipend of $400 was offered. From among the teacher applications, the final selections were made to ensure that the sample of students reflected diversity in socio-economics, rural and urban locations, and ethnicities. The final list of nine schools, from eight different states, is displayed in Table 2.

From these nine teachers’ classes, a total of 710 students participated in the evaluation. Since human subjects were involved, research approval from the University’s Institutional Review Board was sought under Exemption 1. The website’s content is part of regular science curricula that
addresses aspects of the immune system and infectious diseases; therefore, the content of MedMyst is part of regular classroom instruction. All data collected are kept confidential and reported by school number without identifying individual students.

The percentage of students that receive free or reduced lunch (under guidelines set by the National School Lunch Program administered by the United States Department of Agriculture) was used to determine the school’s socio-economic status (Table 2). Students were from grades six (n = 73), seven (n = 539), nine and ten (n = 98). There were nearly equal numbers of females (n = 350, 49.3%) and males (n = 349, 49.2%) with only 1.5% (n = 11) of students not responding to the gender identification question. The students ranged in age from 11 to 17 years (mean = 13, SD = 1.65). The majority of students were Anglo (n = 423, 59.5%), followed by Hispanic (n = 113, 15.9%), African-American (n = 56, 7.9%), Asian (n = 19, 2.6%) and Native American (n = 5, 0.7%). A few students identified themselves as “mixed” (n = 35, 4.9%), while 8% (n = 59) did not identify their ethnicity. The students were representative of varied achievement levels, ranging from “below average” to “above average” as reported by the teachers.

Procedures. Evaluation of the web adventure was independent of other instructional intervention. Teachers were asked to avoid preteaching the content of the website, but after the testing they were encouraged to extend the website content according to their own curricula. All schools were assigned Mission One since it presents the basic concepts that are elaborated in later Missions. Five of the schools were randomly assigned Mission Two and four were assigned Mission Three as indicated in Table 2.

Assessment instruments. The evaluation consisted of four instruments: (i) a pretest for all three missions administered three days prior to playing the assigned adventures, (ii) an opinion questionnaire administered immediately after completing the missions, (iii) a posttest administered 3 days after completing the adventures, and (iv) a teacher feedback questionnaire. Only the student test data are reported in this paper since the hypothesis under investigation focuses on learning gains, rather than preferences or perceptions. While a formal analysis of the attitudinal data is not reported here, it is relevant to the integrity of the scores to provide some insight into the teacher and student responses. Of central importance is whether the students found the materials engaging or whether they dismissed them as boring. The following comments in response to the question, “What did you like most about MedMyst?” are from both teachers and students.

Sample teacher comments:
- “It was interactive and gave students choices and decision points.”
- “I liked the fact that it has quizzes after each activity.”
- “I like using it with my students because they are engaged. When students say to me, “I wish we could learn like this more,” then I am hooked. When they want the website to work on it at home, I know that it is effective!”

Sample student comments:
- “I liked how they turned it into a mystery, that really got me into it!”
- “I felt like a professional agent chosen to be on a mission.”
- “You could interact with objects like viewing slides under a microscope.”
- “It was better than reading and writing things.”

The pretest-posttest instruments normally required 15 to 30 minutes for students to complete. The actual interaction time at the computer for each mission was typically 30 to 40 minutes. The pretest and posttest included 40 multiple-choice science and history content questions (13 from Mission One, 15 from Mission Two, 12 from Mission Three).
Since both the tests contained the same items, the questions were randomized in a different order for the pretest and the posttest. Content questions focused on knowledge of the six infectious agents and their prevention and treatment methods, cause and treatment of cholera, cause and treatment of smallpox, structure and replication of bacteria, structure and reproduction of viruses, epidemiology, and people of historical significance and historical events related to Germ Theory. See Table 3 for a sample of questions contained in both the pretest and posttest.

Teachers were encouraged to play the adventures themselves prior to using them with their students. All teachers were instructed to administer the pretest three days prior to having students play the two assigned missions, allow students to play the two assigned Missions, wait three days and administer the posttest. The three-day time delay between the testing and the use of the web adventure were purposely planned to test the website’s impact on student knowledge without the immediacy of content and exposure.

**RESULTS**

For each Mission a paired t test was performed on the total sample and for each school to determine whether there was a statistically significant increase in scores. Typically, the paired t test is used when the same subjects are measured before and after a process change. Additionally, the effect size was calculated for each pair-wise comparison. Effect size provides a measure of the magnitude of a treatment effect and unlike significance tests, these indices are independent of sample size (9).

**Mission One.** Table 4 shows the pretest and posttest scores, the gain scores, the t test, and effect size (d) for all those subjects who played Mission One. It is clear from Table 4 that students’ performance improved after playing MedMyst Mission One. Across all schools, the gain score was significantly greater than 0, \( P < 0.01 \). Effect sizes of 0.50 are commonly considered medium and those greater than 0.80 are considered large (9). By these standards, the size of the gains appears to be reasonably large for the overall sample and for eight of the nine schools. Only the gains for School 5, seventh grade demonstrated an effect size well below 0.50.

Separate analyses of variance (ANOVAs) were performed for the group of schools that played Missions One and Two and the group that played Missions One and Three. For the former schools, the sizes of the gains varied somewhat as a function of grade. The mean gain scores for sixth, seventh, and ninth grades were 1.93, 2.47, and 1.02. There were no apparent effects of gender. A grade by gender ANOVA found a significant effect of grade, \( F(2,377) = 8.73, P < 0.01 \), but no significant effect of gender or a grade by gender interaction.

For the group of schools that played Missions One and Three, no significant effects were found.

**Mission Two.** As can be seen in Table 5, comparable results were obtained for Mission Two. The only difference is that on Mission Two, School 9 ninth grade students showed virtually no gain. The effect size for School 9 was extremely small 0.15. The gains for the other schools were all significant, \( P < 0.001 \), and the effect sizes were large. As in Mission One, the sizes of the gain varied somewhat as a function of grade. The mean gains for sixth, seventh, and ninth grades were 1.63, 2.03, and 0.31. There were no apparent effects of gender. A grade by gender ANOVA found a significant effect of grade, \( F(2,377) = 18.82, P < 0.01 \), but no significant effect of gender or a grade by gender interaction.

For the group of schools that played Missions One and Three, no significant effects were found.

**Mission Three.** Table 6 shows the gains for Mission Three, all of which were significant, \( P < 0.01 \); however, the effect sizes range from 0.56 to 0.33, which are less impressive than Mission One or Mission Two. A grade by gender ANOVA revealed no evidence of a grade effect, a gender effect, or a grade by gender interaction on Mission Three.

---

**TABLE 2. Demographics of field test population**

<table>
<thead>
<tr>
<th>School</th>
<th>State</th>
<th>Grade</th>
<th>n</th>
<th>Missions Assigned</th>
<th>Economic Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TX</td>
<td>6</td>
<td>73</td>
<td>1 &amp; 2</td>
<td>47%</td>
</tr>
<tr>
<td>2</td>
<td>WA</td>
<td>7</td>
<td>91</td>
<td>1 &amp; 2</td>
<td>8%</td>
</tr>
<tr>
<td>3</td>
<td>PA</td>
<td>7</td>
<td>67</td>
<td>1 &amp; 2</td>
<td>74%</td>
</tr>
<tr>
<td>4</td>
<td>ID</td>
<td>7</td>
<td>84</td>
<td>1 &amp; 3</td>
<td>45%</td>
</tr>
<tr>
<td>5</td>
<td>MD</td>
<td>7</td>
<td>118</td>
<td>1 &amp; 3</td>
<td>39%</td>
</tr>
<tr>
<td>6</td>
<td>CT</td>
<td>7</td>
<td>97</td>
<td>1 &amp; 2</td>
<td>not reported</td>
</tr>
<tr>
<td>7</td>
<td>PA</td>
<td>7</td>
<td>83</td>
<td>1 &amp; 3</td>
<td>33%</td>
</tr>
<tr>
<td>8</td>
<td>ME</td>
<td>9 &amp; 10</td>
<td>36</td>
<td>1 &amp; 3</td>
<td>44%</td>
</tr>
<tr>
<td>9</td>
<td>NM</td>
<td>9</td>
<td>62</td>
<td>1 &amp; 2</td>
<td>11%</td>
</tr>
</tbody>
</table>
TABLE 3. Sample questions from MedMyst pretest and posttest

<table>
<thead>
<tr>
<th>Sample Content Tested</th>
<th>Sample Questions</th>
</tr>
</thead>
</table>
| Infectious agent                    | Prions, viruses, and fungi are three of six kinds of:  
|                                     | a) infectious agents  
|                                     | b) antibiotics  
|                                     | c) vaccines  
|                                     | d) antibodies  
| Cause and treatment of cholera      | A simple treatment for cholera is:  
|                                     | a) oral rehydration solution  
|                                     | b) using a topical ointment  
|                                     | c) taking laxatives  
|                                     | d) cardiopulmonary resuscitation  
| Cause and treatment of smallpox     | Smallpox is caused by a:  
|                                     | a) bacterium  
|                                     | b) prion  
|                                     | c) fungus  
|                                     | d) virus  
| Structure and types of bacteria    | Circle the letter that illustrates a type of coccus bacterium.  
|                                     | a)  
|                                     | b)  
|                                     | c)  
|                                     | d)  
| Structure and reproduction of viruses | Viruses can only reproduce in:  
|                                     | a) air particles  
|                                     | b) dead tissues  
|                                     | c) living cells  
|                                     | d) water  
| Epidemiology                        | When there is an increase in the number of cases of a disease in a particular region, it is called:  
|                                     | a) a toxic level overdose  
|                                     | b) a spirilla  
|                                     | c) an epidemic  
|                                     | d) an infectious disease  
| Historical events/people            | Germ Theory was first proposed by:  
|                                     | a) John Snow  
|                                     | b) Louis Pasteur  
|                                     | c) Joseph Lister  
|                                     | d) Robert Koch  

**DISCUSSION**

The hypothesis for MedMyst evaluation focused on the efficacy of web-based technology in delivering substantive microbiology content to adolescents. The results reported here indicate that MedMyst was successful in supporting students’ learning. The investment of 30 to 40 minutes yielded gains even with a three-day delay in testing and without accompanying classroom instruction. Finding efficacious materials for middle school students is particularly important since this is normally when interest in science begins to wane. Serving as an alternative teaching tool, MedMyst may even reach students not normally interested in science, as well as those who prefer more interactive and visual learning environments.

The diversity of the student sample and the differences in preexisting knowledge documented in this study represent the range of ethnic, socio-economic, and ability differences among students who might encounter the web adventures. In general, students from middle schools were able to improve their scores based on using the web adventure alone. The gains for the high school students sampled were not as consistent or as large as those of middle school students. This reaffirms that the designers’ intended audience (middle school age students) is the best match for the materials. The genre and the format may not be as appealing to high school students or may be too repetitious of previously learned material.

The experimental design in this study tested the efficacy of MedMyst in a broad range of school settings and independent of any teacher intervention. The investment of time on
task for MedMyst affords some clear learning gains for both genders, particularly with middle school students. With the addition of classroom instruction and relevant hands-on activities, gains in knowledge acquisition could be magnified. To further this end, there are now teacher materials available on the website. These consist of a magazine in pdf format to accompany each mission and hands-on activities from which the teacher may select those most appropriate for his or her classes. The other key feature of the MedMyst series is that it takes relatively little class time. In many instances, students could be assigned the web adventures as homework. With the many pressures of standardized testing and the requirements to march through a specific curriculum, teachers often have little time for “extra” material. MedMyst is a catalyst that can hopefully serve as an introduction or review to microbiology concepts that are part of the National Science Content Standards, leaving class time for hands-on activities. The authors would welcome the use of MedMyst

<table>
<thead>
<tr>
<th>Mission 1</th>
<th>Overall</th>
<th>Sch 1/Gr 6</th>
<th>Sch 2/Gr 7</th>
<th>Sch 3/Gr 7</th>
<th>Sch 4/Gr 7</th>
<th>Sch 5/Gr 7</th>
<th>Sch 6/Gr 7</th>
<th>Sch 7/Gr 9&amp;10</th>
<th>Sch 8/Gr 9</th>
<th>Sch 9/Gr 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=710</td>
<td>5.80 (2.24)</td>
<td>5.71 (1.83)</td>
<td>5.67 (2.33)</td>
<td>5.33 (1.93)</td>
<td>5.29 (1.93)</td>
<td>6.20 (2.46)</td>
<td>5.48 (1.99)</td>
<td>6.12 (2.54)</td>
<td>7.50 (2.25)</td>
<td>5.65 (2.18)</td>
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</table>

Pretest (SD)

<table>
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<tr>
<th>Mission 2</th>
<th>Total</th>
<th>Sch 1/Gr 6</th>
<th>Sch 2/Gr 7</th>
<th>Sch 3/Gr 7</th>
<th>Sch 4/Gr 7</th>
<th>Sch 5/Gr 7</th>
<th>Sch 6/Gr 7</th>
<th>Sch 7/Gr 9</th>
<th>Sch 9/Gr 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=390</td>
<td>5.42 (2.09)</td>
<td>4.88 (1.75)</td>
<td>6.11 (2.41)</td>
<td>4.88 (1.78)</td>
<td>5.23 (1.94)</td>
<td>5.70 (1.94)</td>
<td>5.97 (2.07)</td>
<td>6.27 (2.07)</td>
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</tr>
</tbody>
</table>

Posttest (SD)

<table>
<thead>
<tr>
<th>Mission 3</th>
<th>Total</th>
<th>Sch 4/Gr 7</th>
<th>Sch 5/Gr 7</th>
<th>Sch 7/Gr 7</th>
<th>Sch 8/Gr 9&amp;10</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=320</td>
<td>5.87 (2.29)</td>
<td>5.21 (2.08)</td>
<td>5.53 (1.94)</td>
<td>5.95 (2.36)</td>
<td>8.33 (2.10)</td>
</tr>
</tbody>
</table>

Pretest (SD)

<p>| | | | | | |</p>
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<tr>
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</thead>
<tbody>
<tr>
<td>n=84</td>
<td>6.81 (2.66)</td>
<td>6.10 (2.78)</td>
<td>6.62 (2.37)</td>
<td>6.73 (2.69)</td>
<td>9.28 (1.75)</td>
</tr>
</tbody>
</table>

Posttest (SD)

<p>| | | | | | |</p>
<table>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>n=118</td>
<td>0.94</td>
<td>0.88</td>
<td>1.09</td>
<td>0.78</td>
<td>0.94</td>
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</tbody>
</table>

Gain

<p>| | | | | | |</p>
<table>
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<tr>
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<th></th>
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<th></th>
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<tbody>
<tr>
<td>n=83</td>
<td>0.41</td>
<td>0.42</td>
<td>0.56</td>
<td>0.33</td>
<td>0.45</td>
</tr>
</tbody>
</table>

TABLE 4. Mission One scores on 13 item test

TABLE 5. Mission Two scores on 15 item test

TABLE 6. Mission Three scores on 12 item test

*p<0.01  **p < 0.001
by universities or individual microbiologists in their school outreach programs. A previous paper provides middle school science and health teachers with an overview of how to integrate MedMyst adventures into classroom instruction (18). Some of the obvious advantages of this tool are that it is free and available at any time or from anywhere on the web. Future plans include the creation of more missions to cover additional infectious agents and translating the website into Spanish. The comparison of web adventure learning with an equal investment of time on other presentation modes offers more research questions for exploration.

ACKNOWLEDGMENTS

This work was supported by a Science Education Partnership Award (R25-RR15295) from the National Center for Research Resources, National Institutes of Health.

We would like to thank all the teachers and students who participated in the field tests. In addition, a debt of gratitude is owed to our scientific advisors: Major Bradshaw, C. J. Peters, Robert Tauxe, and Joseph McCormick for serving as vital members of our scientific advisory team. Thanks also to Lynn Lauterbach and Liliana Rodriguez for their help in preparation of the student and teacher materials.

REFERENCES


Learning Partnerships Between Undergraduate Biology Students and Younger Learners

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Bates College Department of Biology, Carnegie Science, 44 Campus Avenue, Lewiston, Maine 04240

In two upper-level elective biology courses and one beginning-level general biology course, college students participated in Learning Partnerships with middle or high school classes to study some aspect of biology. The goals were to enhance learning by providing resources to middle and high school students and teachers and by encouraging college students to consider teaching as a learning tool and a possible career goal. The college students designed lessons, activities, and laboratories that were done at the schools and at Bates College. Feedback and data suggest that the partnerships have helped teachers enrich their curricula, enhanced student learning, encouraged additional high school students to consider applying to college, and encouraged college students to consider teaching science.

The idea for partnering college students with younger students as a pedagogical strategy builds on the notion that you learn something best when you have to teach it. Including a science education outreach component in undergraduate science courses gives college students an opportunity to teach what they are actively learning, as well as what they have learned in the past. It encourages them to integrate what they have learned into larger contexts and helps them better understand different perspectives and learning styles including their own (3, 4, 5). This kind of experience also allows them to experience teaching, in the hope that they will consider staying involved with science education at some level.

High school and middle school science teachers are more often being expected to use technology in their classrooms, teach new content and concepts, and cross disciplinary lines that are not familiar to them. Partnering teachers and their classes with college students brings help and fresh energy that can be used in many different ways. Connecting college students with middle school or high school classes also provides opportunities for intellectual, social, and interpersonal interactions between groups of students who might not otherwise cross paths. This in turn supports the social nature of learning that we have come to realize is so important to its success (4). This type of partnership can also offer high school teachers help with meeting requirements to align their curricula with state-mandated learning standards (17, 18).

These “Learning Partnerships” inherently include some of the models that are beginning to transform science education, which are based on how individuals actually learn about science and come to understand the content, skills, and processes necessary to “do” science (7, 12, 15, 16, 19). These models include student-active teaching (15), cooperative and collaborative learning (2, 13), team-based learning (9, 14), active learning (10), experiential learning (6, 7) and service learning (1, 8, 11).

Bacteriology and Virology are junior- and senior-level electives that count toward the Biology major. Learning and Teaching Biology is a beginning-level course that includes majors and nonmajors. Virology does not include a formal laboratory, and for several years I have given students the option of proposing and doing a project of their own design, which can be laboratory-based or not (L. Abrahamsen, Abstr. 16th Annu. Meet. Am. Soc. Virol., abstr. P13-1, p. 214, 1997). I designed Learning and Teaching Biology around required partnerships to focus on teaching as a learning strategy. With experience, it became evident that the partnerships were most successful when college students met with school teachers ahead of time to brainstorm, further develop their ideas, and plan any activities. This allowed careful tuning of the program so that it could be integrated into the teacher’s curriculum.

METHODS
These Learning Partnerships included 11 teachers in 12 partnerships (one teacher participated in two consecutive years), 43 college students, and 146 high school and middle school students, 106 of whom provided responses to evaluations. Participating schools were Bates College, Martel School, Lewiston Middle School, Longley School, and Lewiston High School in Lewiston, Maine, and Edward Little High School in Auburn, Maine. All of the middle and high schools are public schools. There were eight middle school and four high school partnerships.

Logistics of the partnerships. The partnerships in Learning and Teaching Biology were required, while those in Virology and Bacteriology were optional. Many of the partnerships were arranged through the Bates College Center for Service Learning. Some of the participating school teachers had attended a science education outreach workshop and expressed an interest in having their classes learn about particular aspects of biology or microbiology.

In one case, students had worked with a teacher in another context and proposed the new partnership, and in another case, a teacher heard about the partnerships and asked to be included. Their college partners contacted all of the teachers at the beginning of the semester and arranged to meet to talk about how the partnership would progress.
In the college classrooms. In Learning and Teaching Biology, the Partnerships counted as 40% of each student’s grade. In Virology and Bacteriology, students were allowed to decide individually whether their participation would count as 0 to 40% of their grade. In all classes, college students worked in teams of two to five. In the upper-level courses, all participating students were required to present their work at the annual Bates College student research symposium in the form of a talk or poster. Assessment of each Partnership was based on a proposal and bi-weekly progress reports, a written presentation of the entire project with accompanying lesson plan, overall participation, a self-assessment of learning, a peer assessment of each group member’s contribution to the Partnership, a teacher assessment of the group’s work, and (in the case of the upper-level classes) the quality of their final symposium presentation. Not all of the college students participated in classroom visits. Some prepared labs or other materials, participated in assessment, or ran labs when young students came to the College. I met with student teams weekly and on an as-needed basis. Often these meetings were brief and I kept track of what was going on via the progress reports. I attended all of the laboratory sessions that took place at the College. I also attended several classroom sessions when I was invited and available.

In the school classrooms. After meeting with their teachers and planning their programs, students visited their classes on a mutually acceptable schedule that averaged about five times during the semester. Various programs included mini-lectures, investigational labs at the school or the College, small group discussions of the scientific method and experimental design, guided inquiry-based lab experiences, and multi-media presentations such as films. The younger students were required to participate in the Partnerships. They often worked in groups of three or four for laboratory-type experiences, and groups were required to make posters in scientific format to present their work. The college students and their partner teachers assessed the learning of the younger students with tests, quizzes, homework assignments, informal question and answer sessions, journal entries, surveys, and evaluation of the quality of the posters.

An Example from Bacteriology, “Bacteria in Your Environment,” which includes a sample lesson plan is presented in Fig. 1. The general outline for the lesson plans was developed with the help of colleagues in the Bates College Education Department. It is an example of an outcomes-based curriculum format in which the goals are stated first and are used as a focus for the design of the lesson. Students were provided with the headings of the lesson plan (lesson title, outcomes, concepts to be learned, etc.) and were required to complete the plan with their specific information. An example from Learning and Teaching Biology, “An Introduction to Cells and How We See Them,” is presented in Fig. 2.

Evaluations—college and younger students. Participating college students were asked to respond to four questions that were included in an instructor-administered evaluation of the course (Tables 1 and 2). Younger students were given the same questions on an evaluation form that they were asked to fill out at the end of the Partnership (Table 3). Students were asked to respond “yes” or “no” and to provide comments. College students were also asked to assess the contributions of their group members compared to their own contributions by stating whether their peers had contributed “as much,” “less,” or “more” than themselves.

Evaluations—teachers. Teachers were asked to score Bates student preparation, Bates student professionalism, the scientific accuracy of any student presentations, and their own students’ interest in the program as “poor,” “fair,” “average,” “good,” or “great” (Fig. 3). They were also asked to answer “yes” or “no” to three questions (Table 4).

Presentation of the partnerships. Every year Bates has a college-wide research symposium in which students from all disciplines present their research. In Bacteriology and Virology, students who participated in the Learning Partnerships were required to present their work at this symposium. This encouraged students to gather and analyze data of various kinds, to think about their own work in light of other scientific and/or educational research, and to plan and accomplish assessment of their work. Often the “results” section of the college student presentations included the work of their younger Learning Partners, who were also invited to the symposium with their parents and teachers.

RESULTS

College students. The results of the college student evaluations of the Learning Partnerships are shown in Table 1 for Bacteriology and Virology in which the partnerships were optional, and in Table 2 for Learning and Teaching Biology which had required partnerships. An average of 98% of all students reported that the experience of working in a group was a good one. An average of 96.5% learned a significant amount of biology from participating in the Partnership, and an average of 91% said that they would remember the content of the lesson they taught better than had they only studied the material for an exam. Overall, the students in both groups responded very favorably to the Learning Partnership experience, and 98% of them considered the experience valuable.

In all three courses, students were asked to evaluate the work of their group members by comparing the contributions of each group member to their own. Overall, in 80% of cases, students thought that the work of group members was equal to their own; in 8% of cases, students thought that peers contributed less than themselves; and in 12% of cases, students rated the contributions of their group members as greater than their own.

I was interested in finding out whether participation in a Learning Partnership encouraged students to consider teaching science. Before participating, 18% of all students said they would consider teaching science as a career. After participating in a Learning Partnership, this number rose to 36%.

Younger students. The results of responses from 106 middle school and high school students who were asked to evaluate their participation in the Learning Partnerships are shown in Table 3. The responses suggest that these stu-
Lesson Plan Outline

**Lesson Title:** Bacteria in Your Environment

**Outcomes:** What will your students know and/or be able to do at the end of your lesson?
- Define what bacteria are, where they are found, and what they require to reproduce.
- Design a simple experiment using the scientific method.
- Perform a Gram stain and use a microscope to observe bacteria.

**Concepts to be learned:** What concepts do you want your students to understand?
- Growth by binary fission. What constitutes a cell, and what is the difference between a prokaryotic and eukaryotic cell?
- What is a control? What is microscopic magnification? How do we calculate magnification? Which bacteria make you sick, and which ones keep you healthy?

**Curriculum Reference:** How do these concepts/skills connect with the curriculum currently being used?
- The students are learning about cells and organelles. This will give them an example of another kind of cell. They are also learning about health and disease next term, so the idea that bacteria can cause infectious diseases will introduce them to that unit. They are learning about size and scale in math class, so the microscope lab will tie in as well. We will teach them about magnification and how to calculate total magnification for the lenses of a light microscope.

**Maine Learning Results Reference:** Which science/technology/health learning results include these concepts?
- Science and Technology: Standard A: Classifying Life Forms
- Standard B: Ecology – finite resources and populations
- Standard C: Cells and Organelle – preparation and examination of microscope slides, identification of causes and effects of disease, structure and function of cells

**Exploring what students know:** How will you find out what your students already know about your topic? Is there an activity you can use to find out? Can you establish why your concept is relevant to their lives?
- Students will be divided into groups of four. Students will be asked what they know about cells and bacteria. Each group will work together to draw and label an animal cell, and a bacterial cell. The concepts of normal flora, infectious diseases and how bacteria are beneficial in the environment will be introduced.

**Presenting new information:** How will you present your lesson? Is there a way to appeal to different learning backgrounds and styles?
- We will use 4 mini-lectures (bacterial anatomy, replication, growth parameters and cell wall structure) with appropriate overheads and pictures. We will discuss all concepts that are presented in our small groups. We will also talk about the scientific method, and help students to design experiments by proposing a hypothesis and finding ways to test it. We will use experiential learning in lab to teach students microscopy skills and how to make Gram stains. We will ask students to draw what they see, explain what they have learned to their parents (see homework) and explain what they have learned to the people who come to see their posters to facilitate visual, experiential and auditory learning.

**Practicing, applying and/or processing learning:** Once you have presented the new information, how will your students use it?
- Students will use what we have taught them as they design their experiments, collect their samples and do staining and microscopy in the lab. They will use their new knowledge to explain their work to their teachers and peers.

**Assessment/Closure:** How will you know that your students have understood the concepts? How will you test whether they have learned the skills? Can your students understand/do what you set out to teach them?
- We will have students do 2 homework assignments. One will be to write a journal entry explaining how they asked a friend or relative what they already know about bacteria, and describing how they taught them something new. The other homework assignment will be to write the materials and methods section for their poster individually so they can see whether their group mates could reproduce their work by reading the section (they will be able to share and combine their homework for the real posters).
- We will ask students to do Gram stains and focus the microscopes in lab so we know that they can do it. Our material will be included in their next mid-term exam, and we will ask for the results. We will also assess how much our students have learned by observing and listening to their poster presentations.

**Attachments** (not included here – college students were required to provide references, a resource list, a glossary, and examples of student work as part of their final lesson plan.)

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**Format:** College students met with a tenth grade class four times. In the first session at the high school, four college students gave mini-presentations (see below). Groups of four high school students led by a college student mentor used the scientific method to propose a hypothesis about what bacteria they would find in a particular environment, and designed an experiment using agar plates and swabs to test it. The second session was devoted to the collection of bacterial samples in the environments the groups had chosen (the classroom, the art room, the cafeteria, the principal’s office, the bathroom and a student (his normal flora). For the third session, the students met at the bacteriology lab at the college to use stains and microscopes to morphologically analyze the bacteria they isolated. They took pictures of their stains and plates, and counted and categorized the bacterial types they found in each environment. The mentors helped the younger students analyze their data and explained how to present their work in scientific poster format. The students made posters in class with the help of their teacher, and presented them to the college mentors, their teachers and peers at the fourth session at the high school.
Format: College students planned this Partnership as a 5-session unit on cell biology. Each class session was 80 minutes long. They collaborated with a sixth grade teacher to determine the content. They brought microscopes to the middle school, and later helped the teacher write a successful grant to obtain a microscope for her classroom. The college students handed in detailed lesson plans for each of the five days. The teacher still uses the unit as part of her curriculum, and has shared it with colleagues at another middle school.

Session 1
Introduction to Microscopes: Using light and dissecting microscopes we will give each student an opportunity to become comfortable and enjoy using the microscopes. We will give a brief introduction on what microscopes do and how to use them (and how not to). We will then break into 4 groups of 6 students and spend the rest of the class period using the scopes. Each scope will have something different to look at (single celled organisms, blood cells, bread mold, etc.), and each student will be asked to draw what they see. We will spend 15 minutes at the end of class reassembled as a large group, and will ask students to share what they saw and what they thought about working with microscopes.

Session 2
Introduction to Cells and Their Parts: We will introduce plant cells and animal cells and their most important organelles. We will talk about cells as small units of life, and the concepts of single versus multicellular organisms. We will talk about what people need to live, and then draw connections between what we need and what single-celled organisms need. We will spend the second half of the class helping our groups make models of cells using beans, noodles, straws, Popsicle sticks, paper, plastic bags, etc.

Session 3
Tissues: We will talk about how there are different kinds of specialized cells that work together to form tissues and organs. We will use Legos to demonstrate this concept. We will spend the second part of the class looking at tissue slides under the microscopes and making cheek cell slides stained with methylene blue. We will have the students draw and describe what they see.

Session 4
Other Types of Cells: We will talk about other kinds of cells (plant and prokaryotic). We will look at pond water and onion cells, under the microscopes, and talk about how these cells are the same and different from each other.

Session 5
Assessment: We will spend the first part of class administering a quiz on what we have taught the students about cells. We will spend the rest of the class helping students put the drawings they have made together into booklets, and helping them complete any unfinished drawings. The book will be counted more heavily than the quiz when the teacher calculates their final grades on this unit.

FIG. 2. An example of a Learning Partnership in Learning and Teaching Biology entitled “Cells and How We Look At Them.” The course is a beginning-level majors and nonmajors course that satisfies a general education requirement. First-year students comprised about half of the class.
students liked participating in the Learning Partnerships and found their participation an effective way to learn biology.

In the example presented in Fig. 1, high school students were given a pretest at the beginning of the Partnership to assess what they knew about bacteria, microscopes, and the scientific method. Students scored an average of 27% on these tests. When similar questions were asked as part of the next midterm exam after all four sessions of the Partnership, students scored an average of 86% on that portion of the test.

We polled the participating high school students before and after they participated in the Partnerships regarding whether or not they were considering applying to college. Before the Partnerships, 55% said they were considering college. After the Partnerships, 74% reported that they were considering college.

Teachers. After completing the Partnerships, all participating teachers were asked to respond to the questions presented in Table 4. All of the teachers evaluated the Partnership as useful to their students’ learning. Ninety-one percent considered the Partnership worth their time and effort, and 100% said they would be willing to participate in a Partnership again.

Teachers were also asked to evaluate the work of the Bates students and the interest of their own students in the Partnerships on a scale of “poor” to “great” (Fig. 3). In general the teachers were pleased with the performance of the Bates students and with the quality of the lessons they delivered. The teachers also reported a high interest level among their own students.

DISCUSSION

In order to assess the value of Learning Partnerships between college and younger students in the context of an undergraduate class, it is important to consider whether this type of exchange provides educational or other benefits for students. Is this kind of partnership beneficial enough to warrant the considerable time and energy spent? At this point it is somewhat difficult to assess the outcomes quantitatively. Without control groups of middle and high school students, it is not possible to determine the exact contribution that the Partnerships make to the learning of specific content. To address this issue I have begun to work with one of the high schools to use Learning Partnerships for some units of their integrated curriculum while teaching the same material to parallel groups of students using more conventional methods. Nevertheless, the data gathered so far and our collective observations suggest that Learning Partnerships are valuable to college students, teachers, and younger students.

Most of the participating college students considered the partnerships very valuable to their own learning. On av-

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was the group learning format a good experience for you?</td>
<td>100</td>
<td>• Absolutely—it was like a study group.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• I don’t think there is any other way to learn science.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• What one of us didn’t think of, another did.</td>
</tr>
<tr>
<td>Do you think you learned a significant amount of biology from</td>
<td>100</td>
<td>• I learned more about the stuff I already knew.</td>
</tr>
<tr>
<td>the Learning Partnership you participated in?</td>
<td></td>
<td>• Yes, because we had to think about questions we might be asked, and know enough to answer them!</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• We were helping our teacher, so we had to know what we were talking about.</td>
</tr>
<tr>
<td>Do you think you will remember the content of the lesson you</td>
<td>93</td>
<td>• Learning for a test you forget material you don’t use.</td>
</tr>
<tr>
<td>participated in better than you would have if you had only studied it for</td>
<td></td>
<td>We USED this material.</td>
</tr>
<tr>
<td>an exam?</td>
<td>7</td>
<td>• Yes because I studied it more.</td>
</tr>
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<td></td>
<td></td>
<td>• I learned it differently—in a more meaningful and connected way.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• I’ll remember this stuff for a long time!</td>
</tr>
<tr>
<td>Overall, was the Learning Partnership a valuable experience for you?</td>
<td>100</td>
<td>• Yes—I learned how hard it is to teach!</td>
</tr>
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<td></td>
<td></td>
<td>• We wished we had met college students when we were in high school, so we felt this was very</td>
</tr>
<tr>
<td></td>
<td></td>
<td>worthwhile.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• We were surprised how hard our students worked to make us proud of them.</td>
</tr>
</tbody>
</table>

*Percentage of students, *n = 15.*
TABLE 2. College student evaluation responses and selected comments from students in Learning and Teaching Biology who participated in required Learning Partnerships

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was the group learning format a good experience for you?</td>
<td></td>
<td>• For the most part, yes. It is easier to learn together.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• I liked having education majors in my group.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• I especially liked that we all did what we were good at.</td>
</tr>
<tr>
<td>Do you think you learned a significant amount of biology from the Learning Partnership you participated in?</td>
<td></td>
<td>• I learned the content in AP biology, but I saw it differently for this.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Yes—I hated science and now I like it because I see that it is hard for everyone.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• I don’t know, but it sure was fun!</td>
</tr>
<tr>
<td>Do you think you will remember the content of the lesson you participated in better than you would have if you had only studied it for an exam?</td>
<td></td>
<td>• I’m not sure, but probably.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Yes because as we planned our lab, we thought of many more things that were important to help others understand better.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• This is so different from learning for an exam, but I know I will remember it better.</td>
</tr>
<tr>
<td>Overall, was the Learning Partnership a valuable experience for you?</td>
<td></td>
<td>• This was valuable in so many ways.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• I never thought I would like teaching, but I loved this.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Yes—I used to hate science, and now I almost like it.</td>
</tr>
</tbody>
</table>

*Percentage of students, n = 28.*

average, more than 90% of all participating students answered “yes” to questions designed to assess student perceptions of the value of their participation (Tables 1 and 2). Not surprisingly, the percentage of “yes” answers to every question was slightly higher in the group who volunteered to participate compared to the group for whom participation was mandatory, but most responses were positive. It is possible that the students who chose to participate in the former case, or chose to take the course in the latter, were also likely to consider the experience valuable.

All the participating students spent a great deal of time on their Partnerships. They reported spending an average of 4 to 5 hours a week planning, preparing, or delivering lessons. This time was all out-of-class time and certainly could have been spent studying material for exams, but all of the participating students felt that they gained as much or more from participating in the partnerships as they would have from only studying course content. Many participants suggested that the opportunity to test their own knowledge, concepts, and skills in a forum that allowed modification and improvement over time ensured that they would retain far more than they had learned the same material in more traditional ways. I wondered whether spending time on the Partnerships actually took away from study time, so in the upper-level courses I compared the grades on the final exams of the students who participated to the grades of those who did not. Students who participated received the same average grades on the final exams as those who did not.

There were differences between the level of involvement I was required to have in the Partnerships depending on whether the participating college students were junior and senior science majors or less experienced students. The upper-class students with more science experience required help, but not as much help with content. The less scientifically experienced and often younger college students required quite a bit more help with scientific content as well as logistics. I was impressed, however, by the level of engagement and motivation that the younger college students developed, and felt that the time I spent helping them understand the content they were putting into their lessons was valuable in many ways. Our meetings allowed these students to ask questions about any of the course content that they did not understand and created a culture of active questioning in and out of our classroom. I got to know these students and have kept in touch with many even though most did not major in science. I also felt that encouraging the younger students to assume responsibility for their own learning early on in their college years through participation in a Learning Partnership set a good precedent, and gave them time to seriously consider and perhaps take courses to prepare for a
It seems clear that the middle and high school students benefited. The Learning Partnerships enriched their curriculum and offered many opportunities for learning and growth. The younger students visited Bates, had undergraduate role models and mentors, used equipment in the college’s labs, had a hand in constructing their own knowledge as they did science in a new context, and presented their work in a public forum. Their level of enthusiasm and academic engagement dramatically increased as the projects went on. The fact that there was an increase in the number of students who said they would consider going to college is certainly a good outcome, and one that can be further assessed as these students progress in their education. Some of the parents who attended the Bates research symposium to see their child’s poster had never been on the Bates campus before. All were given the opportunity to sign up to receive the Bates monthly calendar of events, and many said that they would be more likely to take advantage of concerts and other public events in the future.

The teachers who participated in the Learning Partnerships reported that they looked forward to the Bates students’ visits as much as their classes did. The extra “adults” in their classrooms offered opportunities for small group discussions, lab groups, individual help, and special projects that would not otherwise have been possible. Teachers were enthusiastic about bringing their students to Bates and reported that their students gained a better understanding of the “real” process of science and college life. The lesson plans that students produced were evaluated, revised if necessary, and returned to their teachers to be used in future classes and shared with colleagues. The lesson plans have been collected and made available to other teachers as well. Several teachers invited the Bates students to continue their Learning Partnership after the semester ended. Some Bates students have returned to their original teacher’s new classes from year to year and have introduced other Bates students to their teachers so that new Partnerships have formed.

The Learning Partnership model seems to lend itself to what DebBurman refers to as “Pedagogical Transferability” (7) in that it can certainly work in any discipline, in a variety of contexts. Even in large classes, offering the option of one or a few Learning Partnerships gives students the opportunity to experience teaching and to contribute to the community without an overwhelming time commitment on the part of the professor. Exposing undergraduates to teaching so that they might consider making it their career is worthwhile, especially in light of the shortage of sciences and mathematics teachers.

### TABLE 3. Evaluations and selected comments of high school and middle school students who participated in required Learning Partnerships

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
<th>Comments</th>
<th>n</th>
</tr>
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</table>
| Was the group learning format a good experience for you?                 | ![](https://via.placeholder.com/15) | • I like working with my friends.  
|                                                                          | 92     | • When I don’t know something, somebody else does.  
|                                                                          | 8      | • I liked asking the college students hard questions.                    |
| Do you think you learned a significant amount of biology from the Learning Partnership you participated in? | ![](https://via.placeholder.com/15) | • Yes, the Bates students explained things well.  
|                                                                          | 93     | • We learned that bacteria are everywhere but not all of them make you sick.  
|                                                                          | 7      | • I learned a lot and it was fun!  
|                                                                          |        | • More than ever.                                                         |
| Do you think you will remember the content of the lesson you participated in better than you would have if you had only studied it for an exam? | ![](https://via.placeholder.com/15) | • When we do hands-on things we remember them longer.  
|                                                                          | 88     | • Definitely—I hate exams.  
|                                                                          | 12     | • I will remember how to design experiments—I would probably not remember that for an exam.  
|                                                                          |        | • I will remember what those plates looked like for the rest of my life! |
| Overall, was the Learning Partnership a valuable experience for you?       | ![](https://via.placeholder.com/15) | • I got to see Bates College for the first time.  
|                                                                          | 98     | • The Bates kids rock!  
|                                                                          | 2      | • We got to use really good microscopes that we don’t have in high school  
|                                                                          |        | • Liked learning about what college classes are like.  
|                                                                          |        | • Yes, yes, yes, thank you Bates students!!!!!!  
|                                                                          |        | • Yes, I loved every minute! Even our teacher had a good time! |

*Percentage of students, n = 106.*
FIG. 3. Teacher evaluations of college student performance during Learning Partnerships. Teachers were asked to rank each student group’s preparation for class or lab work (white bars), the professionalism of the group members (stippled bars), the scientific accuracy of all presentations (black bars), and the interest level of their own class (striped bars) on a scale of “poor,” “fair,” “average,” “good,” or “great.” Bars represent the number of teachers giving each response.

TABLE 4. Evaluation responses and selected comments of high school and middle school teachers who participated in Learning Partnerships

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
<th>Comments</th>
</tr>
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</table>
| Was this Partnership useful to your students’ learning? | 100 0  | • Most definitely—they learned about college, college students, and cells all at the same time!  
• Yes. I was amazed what they remembered by the time the exam came around.  
• It was nice to see how my students responded to the Bates students—they were eager to learn!  
• Yes. They could finally see what a real science lab is like. |
| Was it worth your time and effort?            | 91 9   | • At the beginning it was difficult to connect with the Bates students, but we worked it out.  
• Absolutely! The Bates students helped me teach something that I would not have otherwise tackled.  
• Definitely—the break of not having to teach even a few classes gave me some time to think and observe my students in a different way. |
| Would you do it again?                        | 100 0  | • In a minute!  
• Yes, and I hope I can do it again soon!  
• Yes, we are going to do it again next term with a new class of high school students.  
• I wish I could do it every year!  
• Yes. And knowing what we know now, I think it would be even better! |

*Percentage of teachers, n = 11.*
When undergraduates interact with younger learners, both groups gain an appreciation for what they really understand and what they do not know. The partnerships allow college students to explore more science in more ways and to cross disciplinary boundaries. They enjoy the satisfaction of sharing their expertise and experiences and of interacting with younger students and teaching professionals. Teachers appreciate help. Younger learners enjoy the mentoring of a group of students not so much older than they and gain some understanding of what a college education offers. Ideal partnerships are mutually beneficial (1), and partnering college students with middle school and high school classes seems to approach the ideal.

ACKNOWLEDGMENTS

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REFERENCES

A Web-Based Comparative Genomics Tutorial for Investigating Microbial Genomes

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As the number of completely sequenced microbial genomes continues to rise at an impressive rate, it is important to prepare students with the skills necessary to investigate microorganisms at the genomic level. As a part of the core curriculum for first-year graduate students in the biological sciences, we have implemented a web-based tutorial to introduce students to the fields of comparative and functional genomics. The tutorial focuses on recent computational methods for identifying functionally linked genes and proteins on a genome-wide scale and was used to introduce students to the Rosetta Stone, Phylogenetic Profile, conserved Gene Neighbor, and Operon computational methods. Students learned to use a number of publicly available web servers and databases to identify functionally linked genes in the Escherichia coli genome, with emphasis on genome organization and operon structure. The overall effectiveness of the tutorial was assessed based on student evaluations and homework assignments. The tutorial is available to other educators at http://www.doe-mbi.ucla.edu/~strong/m253.php.

With the emergence of high-throughput DNA sequencing, the availability of complete microbial genomes has increased at an accelerated pace. Research institutions such as the Sanger Center, Pasteur Institute, and The Institute for Genomic Research have sequenced and catalogued over 100 microbial genomes, many of which are publicly available via web-based servers.

As educators, it has become increasingly important to train students in classical methods of microbial analysis as well as introduce them to the emerging field of comparative genomics. Complementing traditional methods of microbial analysis and education, the field of comparative genomics introduces students to topics ranging from prokaryotic genome organization to complex metabolic networks.

The availability of completely sequenced genomes has led to the development of a number of computational methods to identify functionally linked genes and proteins on a genome-wide scale. Among these are the Rosetta Stone (2), Phylogenetic Profile (5), conserved Gene Neighbor (1, 3), and Operon (8) computational methods.

The Rosetta Stone method identifies individual genes that occur as a single fusion gene in another organism. For example the Escherichia coli gyraseA and gyraseB genes (both involved in DNA replication) occur as a single fusion gene in yeast, topoisomerase II (2). The Phylogenetic Profile method links genes that have a correlated presence or absence in multiple genomes. For example the E. coli flagellar genes flgL and flgG are both present in a number of motile bacterial species but are absent in nonflagellar microorganisms (5). The conserved Gene Neighbor method identifies genes that occur in close chromosomal proximity in multiple genomes, such as the GroEL and GroES chaperone genes. This conserved organization often reflects the clustering of genes of related function as well as bacterial operon organization.

Lastly, the Operon method identifies genes likely to belong to a common operon based on the nucleotide distance between adjacent genes in the same genomic orientation (6, 8).

All four computational methods can be applied to identify functionally linked proteins on a genome-wide scale (9). These methods can also be used to aid in the inference of protein function for previously uncharacterized proteins. Functional linkages among proteins may indicate proteins that participate in a common biochemical pathway, proteins that physically interact via protein-protein interactions, or proteins that serve related functions within the cell.

In addition to advancements in comparative genomics, a number of web-based servers have been implemented to aid in the investigation of microbial genomes. From genome browsers such as the Pasteur Institute GenoList to comprehensive databases of raw genome sequences such as those at the National Center for Biotechnology Information website, it has become important to expose students to the wide availability of genomic databases and web servers.

We have developed and implemented a web-based comparative genomics tutorial that introduces students to the concepts of the Rosetta Stone, Phylogenetic Profile, conserved Gene Neighbor, and Operon computational methods. Throughout the tutorial, students are exposed to a number of genome databases and web servers that are used to investigate microbial genome organization as well as to identify functional linkages among microbial proteins. The goals of the comparative genomics tutorial are two-fold. First, we have attempted to provide students a strong foundation in the computational concepts and terminology, and secondly, we have tried to expose students to a number of web-based genome resources and databases that they may find useful in future research activities.

In Ronald Owston’s article “The World Wide Web: A Technology to Enhance Teaching and Learning,” he discusses three specific advantages the web provides that can be uti-
lized by instructors to “promote improved [student] learning” (4). He states that the first advantage is that the “web appeals to the mode of student learning” since many students are accustomed to working with computers in their everyday life. Owston comments that the “computer has become an integral part of [the students’] world…and that they thrive on interacting with [the computer].” The second advantage detailed by Owston is that the “web provides for a flexible learning environment…that enables students to take advantage of the wealth of learning opportunities available through the Internet.” Owston notes that this is often utilized by instructors to create a more “project-based” learning environment. The third advantage discussed by Owston is that “the web enables new kinds of learning…that can promote critical thinking and problem solving skills” since projects involving the web often require students to evaluate a variety of data from a variety of sources (4).

In our functional genomics tutorial, we have employed a combined strategy that includes web-based instruction in conjunction with traditional teacher-based instruction. Bruce Tuckman described a related model Active Discovery and Participation through Technology (ADAPT) (10), in which a hybrid method of web-based instruction and traditional teacher-based instruction was used to teach a study skills course at Ohio State University. Tuckman demonstrated that the ADAPT model, which employed both web-based and traditional instructional methods, increased student learning as compared to traditional lecture style instruction alone. Tuckman noted that this hybrid ADAPT model allowed students to become more “actively involved in the learning process,” through a variety of computer-based instructional activities (10).

Since the field of comparative genomics relies heavily on the use of computers and web-based resources, we hypothesized that a web-based tutorial would be an effective method to introduce students to the field of comparative genomics, as well as to teach them how to use a variety of web-based resources and databases to investigate functional linkages among prokaryotic genes. We assess our hypothesis using both student evaluations and student homework assignments, which suggest that our web-based tutorial is an effective method to introduce students to the field of comparative genomics.

**METHODS**

Figure 1 shows the introductory screen of our web-based comparative genomics tutorial. This site can be accessed at http://www.doe-mbi.ucla.edu/~strong/m253.php. The tutorial consists of 25 web pages partitioned into five sections.

We have implemented this tutorial in conjunction with the core curriculum for first-year graduate students in the biological sciences at the University of California, Los Angeles. Each student attended one of five computer-based laboratory sessions in which the instructor presented the material in the tutorial. The average enrollment for each computer-based laboratory was approximately 27 students. The students were each assigned their own computer terminal and proceeded through the initial tutorial along with the instructor. This gave students the chance to ask questions along the way and helped emphasize the concepts covered in the tutorial.

The first section of the comparative genomics tutorial introduced students to the concepts of the Rosetta Stone, Phylogenetic Profile, conserved Gene Neighbor, and Operon methods, while the subsequent four sections involved an introduction and demonstration of the four databases shown in Fig. 2. The first database discussed in the tutorial was the European Molecular Biology Laboratory Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) server (12). The STRING server is used to identify genes linked by either the Rosetta Stone, Phylogenetic Profile, or conserved Gene Neighbor Method. The STRING server can be accessed at http://www.bork.embl-heidelberg.de/STRING, or as a link from our tutorial.

In order to demonstrate the applications of each of the four web servers we chose the *E. coli* otsA gene for demonstration purposes. The *E. coli* otsA protein is involved in the first step of trehalose biosynthesis and catalyzes the biosynthesis of trehalose-6-phosphate from UDP-glucose and glucose-6-phosphate (11). Although the *E. coli* otsA gene was used for demonstration purposes, it was emphasized to students that the methods applied in this tutorial could be applied to any gene of interest.

Using the comparative genomics tutorial, students proceeded through a step-by-step introduction to the STRING server. Each student submitted the query gene (otsA) to the STRING server on his or her own computer terminal. The *E. coli* otsA gene was chosen for demonstration purposes because it is linked to a single gene by the Rosetta Stone, Phylogenetic Profile, and conserved Gene Neighbor computational methods. While the otsA gene demonstrates a simplified example, it enabled students to become familiar with both the computational concepts and the introduced databases. Student homework assignments involved protein linkages of higher complexity.

**FIG. 1.** Introductory page for the web-based comparative genomics tutorial. This tutorial is available at http://www.doe-mbi.ucla.edu/~strong/m253.php.
Using the STRING server the students found that the
*otsA* gene was linked to a single gene (*otsB*) by the Rosetta
Stone, Phylogenetic Profile, and conserved Gene Neighbor
computational methods. The functionally linked gene, *otsB*,
is involved in the second step of trehalose biosynthesis.
*OtsB* is a phosphatase that dephosphorylates trehalose-6-
phosphate to yield trehalose. Figure 3a-c summarizes the
results of the *otsA* STRING query. The *otsA* and *otsB*
genes occur in close chromosomal proximity in multiple genomes
(Fig. 3a) linking them by the conserved Gene Neighbor
method. The *otsA* and *otsB* genes also have a correlated
presence or absence in a number of genomes (Fig. 3b) linking
them by the Phylogenetic Profile method, and *otsA* and *otsB*
occur as a single fusion gene in *Pyrobaculum aerophilum*
(Fig. 3c) linking them by the Rosetta Stone method.

The second database introduced was the Pasteur Insti-
was used to examine prokaryotic genome organization in *E. coli*.
Continuing with the analysis of the *E. coli otsA* gene,
students examined the genome organization of this gene in the
*E. coli* K-12 genome. Students learned to navigate the
GenoList Colibri (*E. coli*) web server by following the ex-
amples illustrated in the tutorial. The genome organization of the
*otsA* gene revealed that this gene overlaps another gene
(*otsB*) by 25 bp. The overlap of adjacent genes in the same
orientation is a common feature of prokaryotic operon orga-
nization (6), and we therefore link these two genes by the
Operon method (Fig. 3d).

The third database discussed in the web-based tutorial
was RegulonDB (7) (http://www.cifn.unam.mx/
Computational_Genomics/regulondb). RegulonDB contains
information regarding a large number of experimentally docu-
dmented *E. coli* operons, as well as computationally inferred
*E. coli* operons. The comparative genomics tutorial details
the navigation of this site.

The final database in the web-based tutorial was the Da-
tabase of Interacting Proteins (http://dip.doe-mbi.ucla.edu)
(13). This database contains a record of thousands of pub-
lished protein-protein interactions, with the majority of inter-
actions identified in yeast. Protein interactions involving the
yeast *otsA* homologue, TPS2, were demonstrated using the
Database of Interacting Proteins.

**RESULTS**

The comparative genomics tutorial was taught during the
second week of the University of California, Los Angeles,
graduate course M253 (Macromolecular Structure). A total
of five tutorial sessions were given throughout the week to
accommodate all students. In order to evaluate the effectiv-
ness of the tutorial, we administered a student evaluation
where students ranked various aspects of the tutorial using a
0 to 9 scale, where 9 indicated strong agreement with the
statement (or a positive response) and 0 indicated strong
disagreement with the statement (or a negative response).
The paper-based, voluntary evaluation was handed out in
class to all students at the end of each tutorial session. A
total of 136 students turned in completed evaluation forms.
The anonymous evaluation form included a question regard-
ing the student’s current educational year and intended ma-
jor, and six questions regarding their personal assessment of
the tutorial (Table 1).

The current educational year of the 136 respondents was
as follows: 71% of respondents were first-year graduate stu-
dents, 14% were second-year graduate students, 5% were
third-year graduate students, 7% were undergraduates, and
3% did not specify their year.

The undergraduate and graduate disciplines of the re-
spondents varied dramatically and included: biology, mo-
molecular biology, biochemistry, cell biology, pharmacology, physiology, bioengineering, computer science, biomathematics, public health, environmental sciences, chemistry, chemical engineering, psychology, neuroscience, cybernetics, and mathematics. Since the educational backgrounds of the students varied dramatically, we had a broad audience in which to assess our web-based tutorial.

The results of the student evaluations are presented in Table 1. Although the students had a broad array of educational backgrounds, with most students in disciplines outside the fields of genomics or bioinformatics, the majority of student responses suggested an overall positive reaction to the tutorial.

The two questions receiving the highest number of positive responses were of great interest to us. First, we wanted to know if students would recommend this tutorial to other students interested in comparative genomics, and second we were interested to see if the students liked the combination of a web-based tutorial and in-class demonstrations. Both of these items received high scores from respondents with a mean of 7.9 and 7.8 and a median of 8 and 8 respectively. This indicated to us that the majority of the students felt this tutorial would be helpful for new students interested in the field of comparative genomics. It also suggested that the combination of a web-based tutorial and in-class demonstrations were well received by the students.

We were also interested to see if the students thought the tutorial examples were clear and concise. This question yielded the greatest standard deviation, and probably reflected the diversity of student educational backgrounds. While the majority of students agreed that the tutorial was clear and concise (median = 8), a small minority of students did have some difficulty following the tutorial. The overall assessment of the tutorial yielded a mean of 7.6. This is quite a positive response, since only a small fraction of the students were specifically focused on a discipline related to that of the tutorial. For the most part, students also felt comfortable navigating the databases and web servers following the tutorial (mean 7.3, median 8), and felt that the tutorial was worth the time and effort they put into it (mean 7.6, median 8).

A 25-point homework assignment was also given to students to complete over a 1-week period. The complete homework assignment, with answers, is shown in Fig. 4. The homework assignment assessed the students’ understanding of the material covered in the tutorial, as well as required students to apply their newly acquired skills to investigate a new set of genes using the various databases and web servers. Since our web-based tutorial was available online, students were encouraged to go back to the web tutorial to reinforce any concepts they may have had difficulty with during the class. The home page of the web tutorial also had links to all of the web servers that the students needed to complete the homework assignment.

Of the 134 students that turned in the homework assignment, 78 students received a perfect score or only missed a single point, 54 students received 23 points, and two students received 21 points. Most students did quite well on the homework, emphasizing that the methods we employed were effective in teaching the students not only the general concepts covered in the tutorial but also enabled them to independently identify functionally linked genes and proteins using the discussed web servers and databases.

CONCLUSIONS

Here we have described an interactive web-based tutorial that we designed to introduce students to the field of comparative microbial genomics. This tutorial complements traditional lessons in microbiology and helps students con-
Homework Questions: (25 points total)

1. Using the Codon webserver at the Pasteur Institute, do you think panD might be in an operon with any other genes? Why or Why not. (2pts)
   Answer: No, panD is flanked by two genes in the opposite orientation

2. Do you see any other genes in the panD region that may participate in a similar pathway as panD (pantothenate biosynthesis). If so, what can you say about the genome organization these genes. (2pts)
   Answer: Yes, panC and panB also participate in pantothenate biosynthesis and are organized in a potential operon since they are in the same orientation and are separated by minimal distance (~12bp).

3. Using the EMBL STRING webserver, answer the following questions.
   a) Does panD occur as a conserved Gene Neighbor (Neighborhood) with any other proteins. (List COG and E.coli K12 gene name. Use confidence cutoff of 0.4) (2pts)
      Answer: Yes, 2 genes. COG0414 (panC) and COG0413 (panB)
   b) Does panD occur as a fusion protein (Rosetta Stone) with any other proteins. (List COG and E.coli K12 gene name.) (2pts)
      Answer: No
   c) Does panD share a similar Phylogenetic Profile (Phylogeny) with any other proteins. (List COG and E.coli K12 gene name.) (2pts)
      Answer: Yes, 2 genes. COG0414 (panC) and COG0413 (panB)
   d) Do these computationally inferred functional linkages make sense in respect to the proteins biochemical functions. (2pts)
      Answer: Yes, they all participate in a common biochemical pathway.

4. Using the EMBL STRING webserver, answer the following questions.
   a) Does leuC (COG0065 ) occur as a conserved Gene Neighbor (Neighborhood) with any other proteins. (List COG only. Use confidence cutoff of 0.4) (2pts)
      Answer: Yes, 4 Genes, COG0066, COG0473, COG0002, COG0140.
   b) Does leuC occur as a fusion protein (Rosetta Stone) with any other proteins. (List COG and E.coli K12 gene name.) (2pts)
      Answer: Yes, 1 gene COG0066 (LeuD).
   c) What organisms do the leuC fusion proteins occur in? Are these prokaryotic or eukaryotic organisms? (2pts)
      Answer: S. cerevisiae and S. pombe. They are both eukaryotic organisms.
   d) Can you hypothesize how a fusion protein may arise in an organism that has two genes in a common operon? (2pts)
      Answer: A fusion protein may arise from a mutation (nucleotide insertion or deletion) in the stop codon of GeneA. If GeneB is in-frame with GeneA then a fusion protein may result.
   e) Does leuC share a similar Phylogenetic Profile (Phylogeny) with any other proteins. (List COG only) (2pts)
      Answer: Yes, 10 genes. COG0066, COG0106, COG0107, COG0118, COG0139, COG0141, COG0473, COG0040, COG0140, COG0002.
   f) Based on the Phylogenetic Profiles, why do you think some organisms have all of these linked genes while others have none? (3pts)
      Answer: The organisms that have all of these genes may need to actively synthesize certain precursors via a common pathway involving these genes, while the organisms that do not have these genes may either acquire the precursors from the environment or may not need the precursors. (Note, it is likely that all organisms will need the precursors since some of them are essential amino acids, ie Leucine.)
sider microbial organisms from a genomic perspective. In addition to providing a foundation in computational concepts and terminology, this tutorial introduces students to a variety of web servers and genomic databases. It is our hope that these exercises will promote critical thinking and independent learning skills, since the resources presented in the tutorial can be applied to investigate a diversity of research projects.

Both the student evaluations and the homework assignments suggest that the web-based comparative genomics tutorial was an effective teaching tool that provided a clear introduction to the field of comparative genomics, as well as taught students the skills necessary to navigate a variety of web-based servers and databases in order to identify functionally linked genes and proteins in prokaryotic organisms. These results further support the use of a hybrid instructional model (10) that incorporates web-based instruction in conjunction with traditional teacher-based instructional methods. The assessment of the homework assignments also supports the notion that the web can promote “critical thinking and problem solving skills” (4) since the homework assignments required students to creatively apply their knowledge to solve a series of problems using a variety of databases and web servers.

It is likely that it will become increasingly important to train students in the field of comparative genomics since the number of sequenced genomes will continue to rise at an accelerated pace. The comparative genomics tutorial presented here is available at http://www.doe-mbi.ucla.edu/~strong/m253.php.

REFERENCES
Teaching Phagocytosis Using Flow Cytometry

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Investigative microbiology on protists in a basic teaching laboratory environment is limited by student skill level, ease of microbial culture and manipulation, instrumentation, and time. The flow cytometer is gaining use as a mainstream instrument in research and clinical laboratories, but has had minimal application in teaching laboratories. Although the cost of a flow cytometer is currently prohibitive for many microbiology teaching environments and the number of trained instructors and teaching materials is limited, in many ways the flow cytometer is an ideal instrument for teaching basic microbiology. We report here on a laboratory module to study phagocytosis in Tetrahymena sp. using flow cytometry in a basic microbiology teaching laboratory. Students and instructors found the flow cytometry data analysis program, Paint-A-Gate® PRO-TM, to be very intuitive and easy to learn within a short period of time. Assessment of student learning about Tetrahymena sp., phagocytosis, flow cytometry, and investigative microbiology using an inquiry-based format demonstrated an overall positive response from students.

Using an experimental approach to teaching about microbes is rare in basic microbiology laboratories for lack of easy-to-use laboratory methods appropriate to undergraduate use. Yet students learn best when actively engaged in the process of intellectual discovery (4, 5, 11). The goals of the laboratory series described in this paper were to use an inquiry-based process in teaching a basic microbiology laboratory exercise and give students ownership of the design, implementation, and analysis of their experimental work. Students formulated a research question that involved the evaluation of phagocytosis of prey objects by Tetrahymena sp. Experimental data files were acquired using a flow cytometer and analyzed using the academic version of a commercial software program, Paint-A-Gate® PRO-TM. Through this process undergraduate biology majors learned about flow cytometry and experimental biology during 5 to 6 hours of laboratory activities.

The flow cytometer is an instrument that uses a laser beam to evaluate the properties of individual cells as they flow through an interrogation point. It rapidly generates an “optical fingerprint” of each cell within the population as it passes through the laser beam. When cells are labeled with fluorescent probes, several parameters of each cell can be determined simultaneously. Since the cells are interrogated at a rapid rate, data on a statistically meaningful number of individual cells can be acquired in a few minutes, saved, and computer analyzed in a two-dimensional plot format. The flow cytometer has been used extensively in the study of mammalian cells. More recently, it has been used to study microorganisms (3, 8). A search of the American Society for Microbiology journals under “flow cytometry” yielded 4,273 citations from 1992 through 2003. Half of these citations (2,262) appeared in the last 3 years (http://www.journals.asm.org). Flow cytometry is presented in modern basic microbiology textbooks, along with microscopy, as a method to characterize microbial cells (12, 13). In spite of the emerging eminence of flow cytometry in microbiology research, we are aware of no published reports describing its use in the microbiology teaching laboratory.

Tetrahymena sp. has been studied in research laboratories using flow cytometry and in teaching laboratories using microscopy (7, 9). As Bozzone reported, this ciliate is ideal for teaching about experimental microbiology using an investigative approach (1). Tetrahymena sp. require no special equipment and are easy to grow and manipulate in pure culture on the benchtop. They are not pathogenic and phagocytize a wide range of prey quite rapidly.

We describe here microbiology laboratory activities using flow cytometry where first time microbiology students design and carry out basic experiments on phagocytosis in Tetrahymena sp. using fluorescent yeast and beads as prey objects. The dynamic aspects of phagocytosis, coupled with putting the student in charge of all aspects of the experimental process, create an ideal learning opportunity (2, 6).

MATERIALS AND METHODS

Microbial and prey preparations. Tetrahymena sp. cultures with known characteristics are available at the American Type Culture Collection (http://www.atcc.org). For most experiments Tetrahymena sp. were grown in a liquid medium containing 2% proteose peptone, 0.1% yeast extract, 0.5% glucose, and 0.1% NaCl under well-aerated (one revolution per second), ambient temperature conditions for 24 to 48 hours. Concentrations were adjusted to approximately 10^7 cells per test volume. Fluorescein isothiocyanate (FITC)-labeled Saccharomyces cerevisae (14) and fluorescent beads (red fluorescent ~2 µm and green fluorescent ~6 µm; Spherotech, Inc., Libertyville, Ill.) were used as prey in feeding studies.

Microscopy. Students observed wet mounts of Tetrahymena sp. using either their laboratory microscopes fitted with phase-contrast objectives or a Zeiss Axioskope 2 fluorescence microscope with camera and computer attachments.
**Flow cytometry.** Data acquisition was performed using a FACSCalibur or a FACScan flow cytometer (BD Biosciences, San Jose, Calif.) set to acquire forward scatter (FSC), side scatter (SSC), and fluorescence (FL1 and FL2) with log amplification using an FL1 threshold set just below the minimum *Tetrahymena* sp. autofluorescence. Depending on the experiment, either 10,000 total prey and *Tetrahymena* sp. events were acquired, or the flow cytometer was set to acquire between 500 and 2,000 *Tetrahymena* sp. and the number of prey varied.

**Analysis program.** Paint-A-Gate® (BD Biosciences, Immunocytometry Systems, San Jose, Calif.) was used by students for computer analysis of data files acquired on the flow cytometer. This program is intuitive and simple to learn. Two-dimensional dot plots are created in which each cell becomes a dot defining two values obtained from the flow cytometer (e.g., relative size and relative fluorescence). The student analyzing the data file chooses a color and encircles the cell population of interest with the cursor. All of the cells in this population appear in the plot in the chosen color (painting) and can be selected (gated) for further analysis.

**Student preparation.** The material presented here was used in an upper division microbiology course for biology majors with concentrations in microbiology, molecular biology, physiology, and organismal biology (botany, zoology, ecology, and evolution), and biochemistry majors at San Jose State University (SJSU). Students were familiar with distinguishing cellular characteristics of *Tetrahymena* sp. and *Saccharomyces* sp., and light microscopy, fluorescence microscopy, and flow cytometry theory from lecture. Skills in basic culture manipulation were developed in earlier laboratory exercises.

**Student activities.** During 5 to 6 hours, students completed three exercises (Workshops 1-3: http://www2.sjsu.edu/depts/Biology/specialprogs/flocyto/html/fc-p05.html) designed to progressively develop a conceptual framework requiring increasing knowledge about experimental microbiology and flow cytometry (http://www2.sjsu.edu/depts/Biology/specialprogs/flow_cytometry.html). Worksheet 1 addressed basic concepts in microbiology, microscopic observation, and cell characterization and required a *Tetrahymena* sp. culture, fluorescent yeast, and microscopes. Worksheet 2 focused on basic concepts in flow cytometry: fluids, optics, SSC, FSC, fluorescence, and dot plots. A computer exercise included in Worksheet 2 introduced the students to Paint-A-Gate® through the use of archived data files. Worksheet 3 (Fig. 1) addressed scientific investigation. Students proposed a question, established an information base about phagocytosis and prey, designed and conducted an experiment, and acquired and analyzed data files. This experiment required using laboratory facilities and a flow cytometer. The complexity of the experiment dictated the size of the student groups. Students working in groups of two to four cultured *Tetrahymena* sp. in the presence of prey (fluorescent-labeled yeast or beads) under specific conditions, acquired their data files using a flow cytometer, and performed analysis using Paint-A-Gate® software.

**Student assessment.** The laboratory curriculum described here was developed as part of an ongoing grant from the National Science Foundation to two of the authors of this paper (J.T.B. and R.K.), and the assessment of this curriculum has been conducted in accordance with the procedures in the grant proposal. Although an assessment comparing the previous way of teaching about protists to our present methodology would be beneficial in assessing the efficacy of using the flow cytometry experiment, this option was not available because the present methodology was immediately integrated into the laboratory curriculum upon funding of the grant. The outcomes that we report here were derived using flow cytometry laboratory experience assessments among the students enrolled in laboratory sections of General Microbiology (Microbiology 101) at SJSU over a 3-year period. Students were asked about their academic level,
where they had taken their prerequisite courses (cellular biology and organic chemistry), previous experience with flow cytometry, instructor competence, facilities and materials, and learning experiences. In all, 230 students responded to the questionnaire.

**RESULTS**

**Student activities from Worksheet 1 on basic concepts in cell characterization.** Essential terms and concepts were presented in about 45 minutes. These included relative size, relative complexity, fluorescence, autofluorescence, fluorescence microscopy, flow cytometry, FSC, SSC, data files, and dot plots. A theoretical experiment was conducted addressing the question "Does *Tetrahymena* sp. ingest yeast?" Students observed a live wet mount preparation of *Tetrahymena* sp. phagocytizing fluorescent-labeled yeast (Fig. 2A and B) and were asked to predict characteristics of the resulting cell populations and draw the positions of the hypothetical cell populations on the graphs in dot plot format (Fig. 2C).

**Student activities from Worksheet 2 on basic concepts in flow cytometry.** A theoretical experiment was conducted and analysis was performed on archived data files from a previously run experiment addressing the question of how long it takes *Tetrahymena* sp. to reach its maximum yeast uptake. Students were asked how the question could be addressed experimentally using a flow cytometer and then to predict how long it takes *Tetrahymena* sp. to ingest yeast. Students were to imagine mixing *Tetrahymena* sp. with fluorescent-labeled yeast for specified times. With help from the instructor, students chose a time point of interest (5 seconds to 20 minutes) and then analyzed the data files to determine the percent of *Tetrahymena* sp. that had ingested yeast for their time point.

This analysis was accomplished by gating the high FSC, high SSC events (red) on the left plot (FSC versus SSC) (Fig. 3A) and then painting the high FL1-FITC (green) events on the right plot (FL1-FITC yeast versus SSC) of Fig 3B. The green number across from the "%" (in this case 64.64) represents the percentage of *Tetrahymena* sp. that had phagocytized fluorescent yeast at 5 minutes. The results for all the time points were compiled and summarized in graphical form during a class discussion (Fig. 3C).

**Student activities from Worksheet 3 on scientific investigation.** Student groups worked to develop possible questions about phagocytosis in *Tetrahymena* sp. that could be addressed using flow cytometry, and each laboratory section chose one question to address in their section's experiment. Below are examples of questions investigated by several student laboratories:

- What is the optimum temperature range for ingestion of yeast by *Tetrahymena* sp.?
- Does the amount of glucose in the medium affect the ingestion of yeast by *Tetrahymena* sp.?
- Does the size of prey affect ingestion by *Tetrahymena* sp.?

An example of a student laboratory investigation of one of these questions is described here.

**Example of Worksheet 3 experiment, protocol, acquisition, analysis and summary of experimental results exploring the question "Does the size of prey affect ingestion by *Tetrahymena* sp.?"** With instructor guidance, students developed an appropriate protocol for this question (Fig. 1) and performed the experiment using beads of two different sizes each with a different fluorescence emission. Students observed a wet mount of their *Tetrahymena* sp.-bead mixture to assess visually the number and size of beads inside *Tetrahymena* sp. (Fig. 4A). They acquired their data files using the flow cytometer (under instructor supervision). The data files were transferred to a computer laboratory equipped with the PaintA-Gate software, and students analyzed their data individually by opening their data files in dot plot format and gating *Tetrahymena* sp. as in Worksheet 2 (Fig. 3A and B).

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**FIG. 2.** Students observed a mixture of fluorescent-labeled *Saccharomyces cerevisiae* and *Tetrahymena* sp. under phase-contrast (A) and fluorescence (B) microscopy displayed on a 15-inch monitor via a video camera. Students identified the cells present and characterized them in terms of relative size (FSC), complexity (SSC), and fluorescence. Students were asked to predict the placement of each cell type (event) on dot plots of FSC versus SSC and fluorescence versus SSC. Example of a student plot where T = *Tetrahymena* sp. and Y = yeast, fluorescent-labeled *Saccharomyces cerevisiae* (C).
FIG. 3. Students determined the percentage of *Tetrahymena* sp. with ingested yeast over time. Students chose a sample time for analysis, opened an archived datafile for their time point in Paint-A-Gate<sup>®</sup>, made two dot plots (FSC versus SSC and FL1-FITC yeast versus SSC), and painted and gated the *Tetrahymena* sp. population (red events) (A). Students then painted the high FL1 events, representing the *Tetrahymena* sp. cells with ingested yeast, on the right plot (SSC versus FL1-FITC yeast) green (B). Students compiled their results to determine the time it takes for *Tetrahymena* sp. to ingest fluorescent-labeled yeast (C).

FIG. 4. An example of student experimental results from investigating prey size preference (2 μm red fluorescent beads and 6-1 μm green fluorescent beads). *Tetrahymena* sp. were fed fluorescent beads (inset) (A). Dot plots (side scatter versus forward scatter and green versus red fluorescence) show the distribution of *Tetrahymena* sp. populations (B). A graphical summary of student data is shown using the means of five to six replicas of each condition (C).
Students then opened a dot plot (Fig. 4B) for two fluorescence parameters (FL1-Tetra and 6-µm green versus FL2-Tetra and 2-µm red) and identified the four possible populations of Tetrahymena sp. cells present.

The microscopic experimental results show that Tetrahymena sp. can ingest 2-µm red fluorescent beads and 6-µm green fluorescent beads (Fig. 4A). The flow cytometry experimental results identified four Tetrahymena sp. populations based on their ingestion of prey (Fig. 4B). The four Tetrahymena sp. populations are (i) those that have not ingested any beads (gray events, 50.15%), (ii) those that have ingested the 6-µm green beads only (green events, 2.33%), (iii) those that have ingested the 2-µm red beads only (red events, 43.06%), and (iv) those that have ingested both the 6-µm green beads and the 2-µm red beads (blue events, 4.46%). In this laboratory section the students elected to perform six experimental replicas. Figure 4C gives a graphical representation of the results submitted by a student indicating that almost 50% of the Tetrahymena sp. ingested beads and that they preferred the smaller beads. The results were discussed in class, and students were asked to submit a summary of the class results in graphical form with their conclusions from the experiment.

**Student learning.** Student perceptions of their learning experiences were assessed using questionnaires after the flow cytometry exercises. Students felt that these flow cytometry experiences had enhanced their ability to understand protozoology, analyze data, and appreciate computer-aided data analysis (Table 1). Responses indicated that students had learned about flow cytometry and microbiology, that instructional and instrument support for the activities enhanced their experience, and that they would like to see flow cytometry incorporated into other classes (Table 2).

**DISCUSSION**

Learning can be optimized when students with sufficient background determine the questions to be addressed, design and conduct an experiment, and analyze and communicate their findings (10, 11). Experiences are best when the results are meaningful and feedback on results is immediate. In many basic microbiology laboratories the laboratory skills and content knowledge of the students, time, and expense limit such opportunities. This is especially challenging in teaching protist microbiology where most published laboratory protocols are limited to microscopic observation. Our experiences in teaching basic microbiology laboratories using phagocytosis in Tetrahymena sp. as a model system and flow cytometry to acquire data, provide a teaching strategy that transcends most of these limitations.

By following Worksheet 1, students observed a living culture of Tetrahymena sp. ingesting fluorescent yeast and used critical observation skills to characterize the three types of cells present (large, complex Tetrahymena sp. cells containing smaller green fluorescent yeast cells, empty Tetrahymena sp. cells, and extracellular green fluorescent yeast cells). Based on these observations, students learned to translate the cell characteristics into relative flow cytometry parameters (SSC, FSC, and fluorescence) and predict the placement of these cells (events) in dot plot format. Both the microscope and the flow cytometer provide essential and unique information about cells.

Using Worksheet 2, students determined the time course of Tetrahymena sp. ingesting fluorescent yeast using archived data files. In doing so, they learned about experimental design and the importance of controls, data manipulation through painting and gating cell populations, and data analysis (percent Tetrahymena sp. with ingested fluorescent yeast) using computer software (Paint-A-Gate™).

In doing Worksheet 3, students designed and executed an experimental protocol, operated a flow cytometer to acquire their data, analyzed their data, and interpreted their results. The students provided the initiative in all aspects of the experiment with the instructor acting as a resource. This experience built on content about protists and yeasts learned from lecture and previous laboratory experiments with culture manipulation and microscopic observation.

Questions designed to measure the students' perception of these laboratory activities were included in the post-laboratory assessment (Table 2). Student perceptions of the flow cytometry laboratory experience clearly indicated that they felt it was a valuable educational experience and that the experience enhanced their ability to understand the subject area being studied, analyze data, and appreciate data analysis. Students agreed strongly that they had learned about flow cytometry and that they wanted to see flow cytometry used in other classes. For each of the 3 years included in the table, the over-all median for the ratings is 5.0 (data not shown). Thus, the evidence presented here demonstrates that students viewed their exposure to the flow cytometry curricula as a highly positive experience.

Flow cytometry laboratory exercises proved to be ideal for teaching about microbes using inquiry-based formats. The flow cytometer provided rapid acquisition of experimental data and was easy for students to operate. The Paint-A-Gate™ analysis software was intuitive. Extracting meaningful data from 10,000 cells for up to four parameters as

<table>
<thead>
<tr>
<th>Question</th>
<th>Student assessment</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>All years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has your experience with flow cytometry enhanced your...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ability to understand biology?</td>
<td>Yes</td>
<td>43</td>
<td>6</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>6</td>
<td>50</td>
<td>2</td>
<td>118 (11)</td>
</tr>
<tr>
<td>ability to analyze data?</td>
<td>Yes</td>
<td>49</td>
<td>0</td>
<td>51</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
<td>51</td>
<td>1</td>
<td>120 (9)</td>
</tr>
<tr>
<td>appreciation for computer-aided data analysis?</td>
<td>Yes</td>
<td>48</td>
<td>1</td>
<td>52</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1</td>
<td>52</td>
<td>0</td>
<td>115 (14)</td>
</tr>
</tbody>
</table>

*a Frequency of given answer.
*b Answer either “No” or “Not applicable.”
Table 2. Responses to questions included on a post-lab assessment to measure students’ perceptions of their flow cytometry laboratory experiences

<table>
<thead>
<tr>
<th>Questions</th>
<th>Responses</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>All years</th>
</tr>
</thead>
<tbody>
<tr>
<td>This class enhanced my general knowledge of flow cytometry.</td>
<td>4.59 ± 0.76</td>
<td>4.33 ± 0.81</td>
<td>4.33 ± 0.88</td>
<td>4.42</td>
<td></td>
</tr>
<tr>
<td>This class enhanced my ability to understand microbiology.</td>
<td>4.27 ± 0.78</td>
<td>4.35 ± 0.40</td>
<td>3.64 ± 1.20</td>
<td>4.09</td>
<td></td>
</tr>
<tr>
<td>The written material for this class was useful.</td>
<td>4.49 ± 0.68</td>
<td>4.46 ± 0.80</td>
<td>4.20 ± 0.87</td>
<td>4.38</td>
<td></td>
</tr>
<tr>
<td>The instructors were well prepared and knowledgeable.</td>
<td>4.69 ± 0.55</td>
<td>4.56 ± 0.83</td>
<td>4.57 ± 0.73</td>
<td>4.61</td>
<td></td>
</tr>
<tr>
<td>The facilities were adequate to support the class.</td>
<td>4.59 ± 0.73</td>
<td>4.44 ± 0.80</td>
<td>4.25 ± 0.89</td>
<td>4.43</td>
<td></td>
</tr>
<tr>
<td>I would like to see flow cytometry used in other classes that study cells.</td>
<td>4.73 ± 0.72</td>
<td>4.61 ± 0.60</td>
<td>4.29 ± 0.90</td>
<td>4.54</td>
<td></td>
</tr>
<tr>
<td>Overall rating</td>
<td>4.56 ± 0.70</td>
<td>4.46 ± 0.80</td>
<td>4.22 ± 0.96</td>
<td>4.41</td>
<td></td>
</tr>
</tbody>
</table>

*Responses on a 5-point scale, strongly disagree (1) to strongly agree (5).  
*Mean and standard deviation of results.  
*Mean.

Presented here was straightforward. Although a flow cytometer represents a substantial capital outlay, the cost per sample is relatively low, and its applicability to any laboratory that studies cells makes its cost justifiable in many cases. Furthermore, we have established a shared-use facility at SJSU. Student laboratories conducted at schools near SJSU can submit their fixed, experimental samples to us for data acquisition, and then the data files are returned to the off-site campus for analysis using their computer facilities. The one drawback is that students from nearby schools are not able to operate the cytometer unless the class comes to SJSU. With low-cost flow cytometers becoming increasingly available, it is likely that they will be a common feature of student laboratories in the near future.

Acknowledgments

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References

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