APPLICATION OF BIOTECHNOLOGY TO SOLVE RELEVANT BIOLOGICAL PROBLEMS PROMOTES UNDERSTANDING OF UNDERLYING CONCEPTS

P.A. BURROWES and G.M. NAZARIO
University of Puerto Rico, Río Piedras, San Juan, P.R. 0093
pburrowe@upracd.upr.clu.edu, gnazario@upracd.upr.clu.edu

Abstract

Although some efforts have been made to modify the curriculum of the *Introductory Biology* laboratories from a passive to a more experimental form, the use of modern biotechnology had not been implemented at our institution. The need to understand the applications of modern technology to real-life situations seems imperative at the turn of the century [1,2]. Because several studies have shown that the study of biotechnology by itself does not increase conceptual understanding, the objective of this research was to determine if the use of biotechnology to solve relevant biological problems increased conceptual understanding among our students. We designed two complex problems: one on the conservation of an endangered Puerto Rican frog, and the other on tropical plant evolution. Two students majoring in Biology-Education participated as research assistants in the design and implementation of the laboratory activities. Graduate biology students who worked as teaching assistants in the laboratories were trained to use equipment and teach the activities. Assessment evidence indicated that students exposed to these experiences: (1) increased biological literacy by understanding the use and application of cutting-edge biotechnology; (2) were able to make connections between organismal and molecular biology; (3) decreased levels of anxiety and insecurity associated with the use of laboratory equipment; and, (4) were motivated to conduct research within and beyond the classroom setting.

Introduction

As the world becomes increasingly scientific and technological, our future grows more dependent on how wisely humans use science and technology. And that, in turn, depends on the effectiveness of the education we receive. With the exploding impact of science and technology on every aspect of our lives, especially on personal and political decisions that sustain our economy and democracy, we cannot afford an illiterate society [2].

Our participation in the 1999 National Association of Biology Teachers (NABT) Conference made us realize that many high school teachers are providing excellent hands-on biotechnology experiences in the classroom. The literature already presents examples of exercises involving biotechnology that have been implemented effectively at the high school level [1,2,3]. This came as a shock to us because the majority of students in Puerto Rico do not have this opportunity in their high schools, or even in first- and second-year biology courses at the university level. Although biotechnology is undoubtedly promoting breakthroughs in the discipline, awareness of the significance of this relatively new branch of science remains limited [1]. A research group led by J.H. Wandersee from Louisiana State University (1996) found that instrumentation and methods drive research even more than theory does. Science laboratories strive to keep up with the latest instruments and methodologies, and their possibility of achieving funding rests in their competitiveness and productivity in applying these techniques [4]. The American Association for the Advancement of Science (AAAS), in *Benchmarks for Science Literacy*, emphasizes the importance of teaching technology to science students because it: encourages development of scientists; fosters development of new lines of research; and, inspires the design of new technology itself [5].

In the introductory biology laboratories (*General Biology*, *Botany*, and *Zoology* courses) at University of Puerto Rico, Río Piedras (UPR-RP), we have not used biotechnology for a variety of reasons; including, financial constraints, lack of experience among teaching assistants, and scarcity of laboratory exercises that deviate from simple "cookbook" recipes. One of the objectives of this research was to address these deficiencies. We designed two biological problems pertinent to Puerto Rico that required students to analyze scientific literature and apply biotechnology to obtain answers. The educational value of this experience was assessed with respect to its potential to increase conceptual understanding and trigger motivation toward scientific investigation. In the following pages, we describe the exercises emphasizing the skills that we aimed to develop among the students.

Methodology

The Conservation of an Endemic Puerto Rican Frog *Bufo lemur* ("sapo concho") - Target: Students in *Zoology* or *General Biology II* — The biological problem is one of conservation of a native species that is endangered. The laboratory exercise consists of a paternity analysis to identify the offspring sired by healthy parents, and thus, use that stock for re-introduction to the wild. *Bufo lemur*, known locally as the "sapo concho," is unique to Puerto Rico, but it is declining drastically, and efforts to reintroduce individuals that have been bred in captivity at several zoos in the U.S.A. have been unsuccessful. Because zoo bred animals are kept in extreme sanitary conditions to prevent bacterial infections, it is likely that their immunity is weak and they succumb easily to natural pathogens in the wild.

A week in advance, students are given a reading assignment of an article authored by a local expert [6]. This article describes the natural history of the "sapo concho" and comments on its conservation problems. At the beginning of the lab, students are divided into small groups to discuss the article. Then, the instructor tells them to suppose that a group of scientists were able to genetically engineer a stock of male frogs with strong immunity that could survive in the wild. At this point, students get very excited and generally remark that what needs to be done is to breed these frogs so that they pass on their "good" genes to their offspring, and then use this stock for reintroduction to the natural habitat. However, the instructor reminds them that it is expensive to engineer these "good" frogs and thus, that their number would not be abundant enough to create the great choruses needed to stimulate females to reproduce. Thus, it becomes apparent that they will have to use "good" and "bad" males to achieve mating, and that the problem will be to identify the offspring sired by the "good" males. Students are led through a series of questions on how biotechnology could contribute data that would be useful in identifying the father of the progeny, and with some guidance from their laboratory instructors, they participate in the design of the methodology to be used.

For the purpose of this lab, we could not extract DNA from the frogs because *Bufo lemur* is an endangered species; so we told the students that we would be using "pretend frog DNA" that had been obtained from bacteriophage lambda. Four different, but related bacteriophage DNA types were purchased from Fotodyne, Inc. (Cat. No. E1-2207/2208), and pre-cut with several restriction enzymes. This provided an opportunity for instructors to explain what restriction enzymes are, where they come from, and their importance in biotechnology. These enzymes have the ability to recognize particular DNA sequences and digest them at specific sites producing unique DNA fragments for an individual. Variation in the length of the DNA fragments (restriction fragment length polymorphisms – RFLP's) are due to the differences in the genetic makeup of individuals, and can be viewed as stained bands when the DNA is separated by processes, such as electrophoresis. Individuals that are more closely related to each other will have more similarity among band patterns. Students were given four tubes with dry DNA, labeled: (1) offspring; (2) mother; (3) potential sire 1 = "bad" male; (4) potential sire 2 = "good" male. The laboratory procedure involved re-hydrating the DNA, centrifuging, loading it onto agarose gels, electrophoresing for 46 minutes, dying the gel with ethidium bromide, and viewing the fragments under a UV transiluminator to observe genetic differences among the samples (Fig. 1). After photographing the gels, students used rulers to help determine the number of shared bands ("fingerprints") between the offspring and the potential sires. From their results, they were able to infer which was the most likely parent of the offspring, and decide whether this stock was appropriate for re-introduction to the wild. Although the DNA that we used was not really *Bufo lemur*, the students actually underwent all the steps of the scientific method and were able to apply biotechnology in the same manner that it would have been applied if we had used the frog DNA. Thus, we do not feel that the use of virus DNA hinders the learning experience that this exercise offers. On the contrary, it made the laboratory simpler, shorter, and more cost effective for teaching purposes.

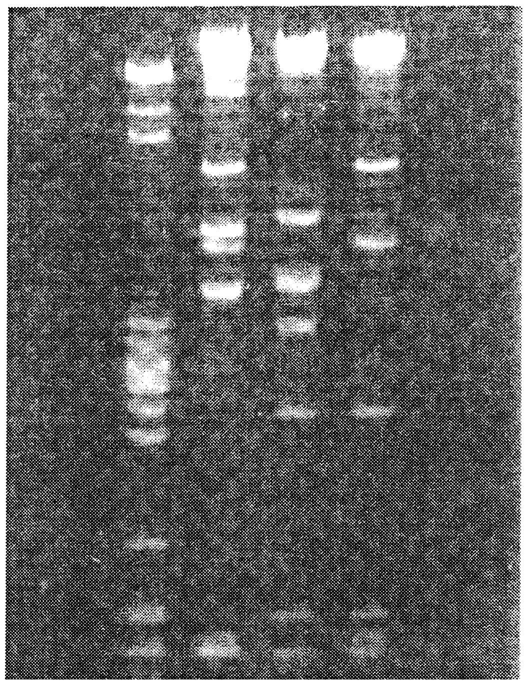

Figure 1. Results of a photograph taken by the students showing electrophoresis results of the frog ("sapo concho") conservation case. From left to right, you can observe the banding pattern obtained for offspring (lane 1), mother (lane 2) and potential sires (lanes 3 and 4).

<u>The Genetic Basis of the Observable Diversity in Tropical Plants - Target: Students in *Botany* or *General Biology II*</u> — This problem makes reference to the fact that plants show anatomical and morphological differences that are based on the genes shared among close relatives. Plants include organisms that range in complexity from bryophytes to angiosperms. Their evolution includes processes, such as the development of vascular tissue, seeds, and flowers. In a laboratory organized in stations, students manipulate the roots, stems, leaves, and reproductive organs of live plants. They use the light and dissecting microscopes to make observations and to determine plant size, presence or absence of vascular tissue, morphology of reproductive structures, and arrangement of parts. Finally, they use their findings to identify and classify the plants in evolutionary sequence. In the next laboratory period, they get to investigate if these morphological differences have an underlying genetic basis.

Instructors take students around campus to collect tropical representatives of three plant groups: ferns (vascular, non-seed), gymnosperms (vascular, seeded, non-flower), and angiosperms (vascular, seeded, and flowering). They extract DNA from each plant group (Fig. 2) using Ward's Isolation and Purification of Plant DNA (Cat. No. 36W5902). DNA samples are amplified with random primers by Operon RAPD 10-mer kits containing 10-base oligonucleotide primers selected randomly from a group of sequences with a 60% to 70% (G + C) content and no self-complementary ends. Amplification reactions are performed in a thermal cycler programmed for 45 cycles of 1 minute at 94^0 C, 1 minute at 36^0 C, and 2 minutes 72^0 C. Amplified DNA fragments are electrophoresed in 1.4% agarose gels for 50 minutes, and detected by staining with ethidium bromide to observe differences in the DNA amplification patterns. Students compare results within and among plants from the different species of ferns, gymnosperms, and angiosperms. By quantifying differences in banding patterns among the groups, they infer evolutionary relationships and decide whether they do, or do not support the present classification.

For both the frog conservation and the plant evolution laboratory experiences, teaching assistants present the basic concepts related to molecular biological techniques, such as DNA extraction, amplification by the Polymerase Chain Reaction (PCR), digestion by restriction enzymes, random priming, electrophoresis, and analysis of DNA fingerprints. Their presentations are enriched by visual aids such as movies, and three-dimensional computer images. In addition, we provided an activity with paper manipulatives representing the DNA molecule and 3'—5' and 5'—3' primers, so that students could do hands-on what a PCR Thermocycler actually does (modified from Fotodyne workshop at NABT, TX, 1999).

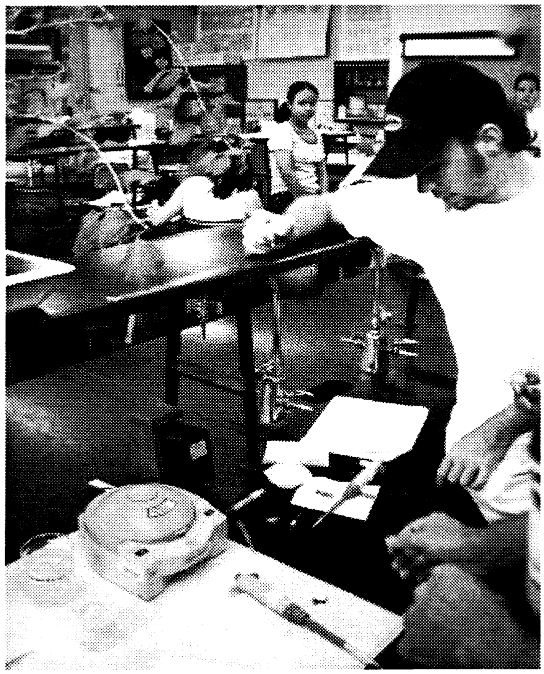

Figure 2. Students working on the isolation of plant DNA.

Results

The application of biotechnology to the two biological problems that we described above included several steps that aimed to develop the following skills:

- Analytical Reading of Scientific Literature – Readings from specific journals were assigned and discussed;
- Critical Thinking – At the end of each laboratory, students analyzed data from molecular markers, drew conclusions, and provided answers to specific questions regarding the problem (Fig. 1);
- Interdisciplinary Connections – Students had the opportunity to integrate concepts from molecular biology and genetics, to ecology, evolution, wildlife management, and conservation;
- Use of Technology – Students learned to use micro-centrifuges, micro-pipettes, PCR thermocycler, electrophoretic chambers, and UV transilluminators (Fig. 2).

The laboratory exercise on frog conservation was offered to 150 students in *General Zoology*, and the lab on tropical plant evolution was given to 156 students in *General Botany*. In order to do this, six graduate students from the Department of Biology, as well as a lab technician, were trained to run the experiments successfully, and to conduct the entire laboratory activities with an inquiry-based teaching strategy. To investigate if this type of exercise promoted understanding, we designed a short test (ten items) that addressed the major concepts of the molecular biology involved in the biotechnology applied. This test was administered in a pre-test/post-test fashion. Student achievement in the post-test was significantly better than in the pre-test (In Zoology: student $T = 4.11$, $P = 0.000$, n = 95; In Botany: student $T = 4.78$, $P = 0.000$, n = 128), suggesting that conceptual understanding increased after performing the exercises. An assessment instrument (Table 1) to evaluate student attitude and perception with respect to this type of experience showed that they enjoyed these labs much more than the traditional descriptive type, and felt more confident about using biotechnology equipment.

Table 1. Percentages of student replies to specific questions that assessed their perception and evaluation of these laboratory exercises. Responses were from 1 to 4; where 1 = strongly disagree, 4 = totally agree.

Laboratory Exercises:	**Sapo Concho**				**Plant Evolution**			
Question	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1. I enjoyed this problem-based lab more	0	3	10	87	0	2	7	91
2. I had time to learn to use the equipment	0	12	9	79	0	13	10	77
3. This exercise has helped me understand	0	0	3	97	0	0	4	96
4. I would recommend my professor	0	0	4	96	0	0	0	100
5. This exercise has encouraged me	4	6	7	83	3	9	11	77

Conclusions

This research aimed to design exciting biological problems that students would resolve, using cutting-edge biotechnology as a tool to increasing conceptual understanding. Other scientists have suggested this practice as a means to develop research skills [7,8]. Our findings showed that both objectives were accomplished, and student evaluations served to motivate us to continue our efforts in this direction (Table 1). Our efforts served not only to contribute to the education of the undergraduate students enrolled in the courses, but also to train graduate students who served as teaching assistants to the labs. These students benefited from learning two aspects essential to their future careers: the application of a research tool; and, constructivist teaching methods. At present, the University of Puerto Rico holds a grant (NSF grant to J. Arce - PR K-12 Graduate Fellowships) that provides our graduate students the opportunity to work closely with cooperating teachers supervising in their classrooms. Our work complements this proposal by providing professional development to graduate students in research and educational strategies.

The three courses (*General Biology*, *Zoology*, and *Botany*) that are enriched by these lab activities serve a heterogeneous group of students, which include Science-Mathematics-Education (SME), and all Natural Science majors. We expect that students exposed to the use of biotechnology in the laboratories will develop a positive attitude toward experimental biology. In addition, we anticipate that the opportunity to use sophisticated biotechnology in a pertinent setting will motivate students to become involved in research, study more, and generally perform better in science courses. ■

Acknowledgments

This research was partially funded by a Collaborative for Excellence in Teacher Preparation (CETP) grant to Josefina Arce, University of Puerto Rico; and, by NASA - Puerto Rico Space Grant Consortium (NASA-PRSGC). We thank the Department of Biology at the University of Puerto Rico, Río Piedras for providing administrative support and several undergraduate students, Miley Oliveras, Daniel Gonzalez, and Carlos Galeano, who served as research assistants.

Bios

Patricia A. Burrowes completed her Ph.D. in Ecology and Evolutionary Biology at the University of Kansas, and Gladys M. Nazario completed her Ph.D. in Plant Physiology and Biochemistry at the University of California, Riverside. They are presently Associate Professors of Biology on the Faculty of Natural Sciences at the University of Puerto Rico, Río Piedras. Their main research interest in the area of education is methods for promoting conceptual understanding in biology.

References

[1] M. Ahmed, "Biotechnology in the High School Classroom," *The American Biology Teacher*, **58**(3) (1996) 178-180.

[2] G.D. Nelson, "Science Literacy for all in the 21st century," *Educational Leadership*, **57**(2) (1999) 14-17.

[3] D. Bowlus and S. Grether, "A Polymerase Chain Reaction Laboratory for Introductory Biology Classes," *The American Biology Teacher*, **58**(3) (1996) 172-174.

[4] J.D. Wandersee, "Preface," in J.D. Wandersee, D.R. Wissing, and C.T. Lange (eds.), *Bioinstrumentation Tools for Understanding Life*, National Association of Biology Teachers, White Plains, MD, 1996.

[5] *Benchmarks for Science Literacy*, American Association for the Advancement of Science (AAAS), Oxford University Press, New York, 1993.

[6] R.L. Joglar, "Se nos extingue el Sapo Concho," in R. L. Joglar (ed.), *Que Cante el Coqui! Proyecto Coqui*, San Juan, PR, 1999.

[7] K.A. Parson, "Recombinant DNA technology: A topics course for undergraduates," *Journal of Chemical Education*, **65**(4) (1998) 325-326.

[8] J. Wagner, "Recombinant DNA paper model simulation: The genetic engineer," *The American Biology Teacher*, **60**(7) (1998) 531-534.