Title

Battle of the Bacteria: Characterizing the Evolutionary Advantage of Stationary Phase Growth

Journal

Journal of Microbiology and Biology Education, 17(2)

ISSN

1935-7877

Authors

Kram, Karin E
Yim, Kristina M
Coleman, Aaron B
et al.

Publication Date

2016-05-01

DOI

10.1128/jmbe.v17i2.981

Peer reviewed

JOURNAL OF MICROBIOLOGY & BIOLOGY EDUCATION, May 2016, p. 261-268
DOI: http://dx.doi.org/10.1128/jmbe.v17i2.981

Curriculum

Battle of the Bacteria: Characterizing the Evolutionary Advantage of Stationary Phase Growth †

Karin E. Kram[1]*, Kristina M. Yim[2], Aaron B. Coleman[3], and Brian K. Sato[4]*
[1]*Department of Biology, California State University, Dominguez Hills, CA 90747,*
[2]*Department of Genetics, Yale University, New Haven, CT 06510,*
[3]*Division of Biological Sciences, University of California, San Diego, CA 92093,*
[4]*Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697*

Providing students with authentic research opportunities has been shown to enhance learning and increase retention in STEM majors. Accordingly, we have developed a novel microbiology lab module, which focuses on the molecular mechanisms of evolution in *E. coli*, by examining the growth advantage in stationary phase (GASP) phenotype. The GASP phenotype is demonstrated by growing cells into long-term stationary phase (LTSP) and then competing them against un-aged cells in a fresh culture. This module includes learning goals related to strengthening practical laboratory skills and improving student understanding of evolution. In addition, the students generate novel data regarding the effects of different environmental stresses on GASP and the relationship between evolution, genotypic change, mutation frequency, and cell stress. Pairs of students are provided with the experimental background, select a specific aspect of the growth medium to modify, and generate a hypothesis regarding how this alteration will impact the GASP phenotype. From this module, we have demonstrated that students are able to achieve the established learning goals and have produced data that has furthered our understanding of the GASP phenotype.

INTRODUCTION

The goal of many STEM curricula is to train students not only in discipline-specific concepts and skills, but also to create a cohort of scientifically literate individuals who are capable of confronting the ever-increasing prevalence of science in society. One of the most obvious yet problematic examples of this is evolution, and the wide variety of views regarding evolution that are present in the population (9). As science ambassadors, we expect our undergraduates to be capable of explaining the impact of evolution on life on planet Earth and describing the many examples of data that support evolution. Despite this, evolution can be a daunting topic to approach in the classroom, and many instructors are unprepared to do so (1, 12).

A common complaint regarding evolutionary theory is that it is not possible to "see" evolution occur (12). This is clearly not the case with microorganisms, as it is very feasible to grow billions of cells with rapid doubling times in a wide variety of environments in order to isolate strains with novel characteristics. Bacterial evolution has been demonstrated in many scenarios. A classic example of this is the fluctuation test performed by Max Delbrück and Salvador Luria using *Escherichia coli* infected by a bacteriophage (8). This experiment demonstrated that mutations in a population occur randomly and in the absence of a specific stress, as bacteriophage resistant cells arose whether or not the phages were present in the culture. Once the mutants resistant to phage were present in the population, the addition of phage acted as a selective pressure, ensuring increased survival of the mutants and leading to evolution of the population.

To provide students with an opportunity to observe evolution, we created a novel microbiology laboratory module that examines the growth advantage in stationary phase (GASP) phenotype (4, 5). The GASP phenomenon is observed in microbes incubated into long-term stationary phase (LTSP – greater than 10 days), which are then better adapted to the environment and thus able to outcompete un-aged cells in fresh medium. This module begins with the premise of mutations occurring during normal growth, which allow for cells to survive the harsh growth conditions of long-term stationary phase. It is discovery-driven, and allows students the opportunity to not only observe evolution in real time, but to participate in the scientific method by

*Corresponding authors. Mailing addresses: Brian Sato: 2238 McGaugh Hall MC3900, Irvine, CA 92697. Phone: 949-824-0661. Fax: 949-824-8551. E-mail: bsato@uci.edu; Karin Kram: 1000 E. Victoria St. NSM A-137, Carson, CA 90747. Phone: 310-243-1090. Fax: 301-243-2350. E-mail: kkram@csudh.edu.
†Supplemental materials available at http://asmscience.org/jmbe

selecting an experimental variable, developing a hypothesis, conducting the experiment, and analyzing novel data.

The goals of this lab module are twofold. First, students can increase our knowledge of the GASP phenotype through an experiment of their design. Second, students will achieve a number of learning objectives (Table 1). Based on a variety of measures of assessment, including a pre-/post-test, self-assessment, course exams, and a pipetting exercise, the module was successfully implemented in two quarters of a microbiology lab at a large enrollment research university. The class-generated experimental data agrees with published literature and contributes novel information, which K. E. Kram's research group is currently analyzing, and the students achieved the established learning outcomes.

Intended audience / Prerequisite student knowledge

This lab module was designed for and implemented in an upper-division microbiology lab course, Biological Sciences M118L, at the University of California, Irvine. This course meets three times each week, including a two-hour lecture that is followed by three-hour and one-hour lab periods later in the week. The course enrollment consists of third- and fourth-year students. While all levels of students could accomplish the lab protocols, it would be beneficial if they have some prior exposure to sterile technique and microbial growth procedures. In addition, experience with micropipettors would be helpful, although many of our students had not previously worked with them. In either case, this module should improve students' pipetting abilities. The conceptual aspect of this module is more challenging than the associated techniques involved, and requires the students to have a molecular biology course, or a molecular biology module in an introductory biology course, as a prerequisite to understand concepts such as transcription, translation, DNA mutations, and the use of selective markers. A microbiology course prerequisite is not necessary, and the instructor can provide appropriate supplemental materials (Appendices 2 and 3) on bacterial culture growth phases, dilution assays, the enzyme catalase, and spectrophotometers.

Another challenging aspect of the module is the hypothesis construction and the requisite literature search necessary to develop an evidence-based prediction. While we believe this is a key portion of the experiment as it allows for participation in the scientific method, this may be difficult for students unaccustomed to searching for and reading scientific articles, and thus can be eliminated if necessary. In our lab course, all four portions of the module were conducted, although this can be adjusted for the specific audience, the course meeting times, and the time available in the course curriculum. More discussion regarding this possibility is included below.

Learning time

The GASP module requires four weeks of the curriculum, including time for students to select a variable, develop a testable hypothesis, and perform the four different protocols to characterize the various aspects of the phenotype. A potential timeline for students to conduct the specific assays in the module is shown in Figure 1. Introduction to the GASP phenotype and how it can be experimentally illustrated can be covered in one hour of lecture time. An additional hour of lecture time would be required to go into further detail regarding the RpoS activity assay, environmental stress measurement, and determination of cellular mutation frequency. It is also recommended that throughout the duration of the module, the instructor provides example data analysis problems (Appendix 3), so that the students are comfortable analyzing their own data once it is collected.

In the week one lab, students work in pairs to start their bacterial cultures. They grow cells in LB media and in media modified by one variable of their choosing.

TABLE 1.
Module learning objectives and methods of assessment.

Learning Objectives	Assessment Method
1. Describe the relationship between mutations, environmental stress, and evolution.	Pre-/Posttest, Self-Assessment
2. Describe the importance of the ingredients in microbial growth media.	Pre-/Posttest, Worksheet
3. Design a testable hypothesis.	Pre-/Posttest, Worksheet, Course Exam
4. Define and describe the use of a transcriptional reporter gene.	Pre-/Posttest, Course Exam
5. Differentiate between bacterial species and bacterial strains.	Pre-/Posttest
6. Differentiate between genotypic and physiological (non-genotypic) changes.	Pre-/Posttest, Course Exam
7. Use micropipettors with accuracy/confidence.	Pre-/Posttest, Pipetting Test, Self-Assessment
8. Perform dilution calculations.	Pre-/Posttest, Pipetting Test, Course Exam

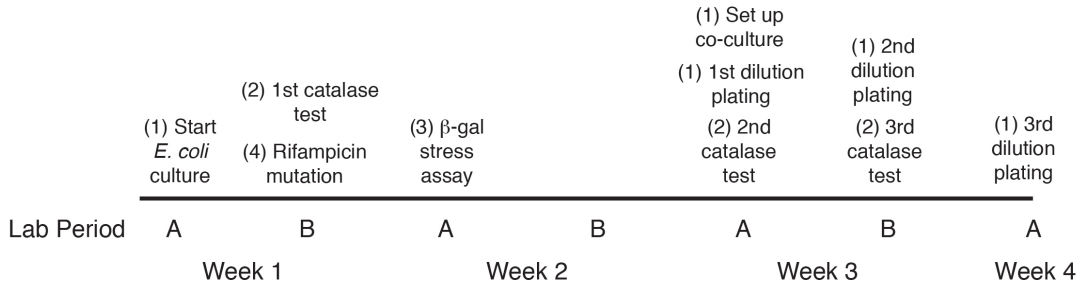

FIGURE 1. Timeline for the four experiments in the GASP module. The module was implemented in a lab course that met twice a week (Lab Period A and B). Lab Period A was three hours in duration while Period B was one hour. Each activity during lab is associated with one of the four specific experiments. (1) Growth of cells into long-term stationary phase and GASP assay, (2) Examination of RpoS activity, (3) Cell stress measurement, (4) Examination of mutation frequency. Specific times required for each activity are indicated on the figure in minutes.

These cultures will grow for two weeks into long-term stationary phase, at which point the GASP experiment will begin. During this two-week growth period, students will assay for RpoS activity, cell stress, and mutation frequency at time points two to ten days following culture inoculation (the day at which these assays are conducted is flexible). In week three of lab, students will set up their co-culture assay, and then measure growth of the aged and un-aged strains a few days and a week later. The specific lab procedures take variable amounts of time, from ten minutes to set up cultures to roughly an hour to conduct the β-galactosidase cell stress assay (Appendix 2). At the end of the experiment, students complete a worksheet (Appendix 4) where they analyze their data, determine the relationship between the GASP phenotype, their variable, mutation frequency, and cell stress, and conclude whether their results support their hypothesis. If their results do not support their hypothesis, they provide a possible evidence-based reason.

PROCEDURE

Materials (separated for each experiment in the module)

1. **Growth of cells into long-term stationary phase and GASP assay**

   Equipment and reagents: Sterile glass tubes (that hold 5 mL culture), Bunsen burner, 37°C incubator, P10, P20, P200 micropipettors, pipette tips, various stock solutions for media variables

   Cells and media: Streptomycin resistant (Strep) *E. coli*, Nalidixic acid (Nal) resistant *E. coli*, liquid lysogeny broth (LB), LB + Strep agar plates, LB + Nal agar plates

   All bacteria can be obtained from K.E.K. or Dr. Steven Finkel (4)

2. **Examination of RpoS activity**

   Equipment and reagents: Sterile glass tubes, microscope slides, 3% hydrogen peroxide, P20 micropipettor, pipette tips

3. **Cell stress measurement**

   Equipment and reagents: Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 50 mM b-ME), Phosphate buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4), 0.1% SDS, 4 mg/ml ONPG, 1 M Na_2CO_3, spectrophotometer, cuvettes, P200, P1000 micropipettors, pipette tips

   Cells and media: LB agar plates

4. **Examination of mutation frequency**

   Equipment and reagents: Empty petri dishes, P20, P200 micropipettors, pipette tips

   Cells and media: LB agar plates, LB + rifampicin agar plates

A detailed list of reagents for all assays, including requirements per student and faculty instructions, are included in Appendix 1.

Student instructions

Students, working in pairs, are provided with the background information and protocol in the lab manual as well as lecture slides (Appendix 2 and 3). The core aspect of the module is to determine whether cells are capable of displaying the GASP phenotype in various media. Students begin by selecting one variable (for example, different sugars, amino acids, or salts) to add to the LB broth and grow two sets of *E. coli* cultures, one in LB and the other in LB plus the variable. After selecting a variable, students will perform a

literature search to generate a hypothesis regarding how that variable will impact the experiment. While they are not provided specific instructions on how to begin their literature search, an excellent starting point would be Farrell and Finkel (2003) and Finkel (2006) (4, 5). Some examples of student-generated hypotheses are presented in Appendix 8. These cultures are grown for at least ten days at which point the cells are diluted into fresh media (again, LB and LB plus variable) that contains an isogenic *E. coli* strain in early stationary phase (~16 h of growth). It is possible to distinguish the aged and un-aged bacterial strains as each possesses a distinct antibiotic resistance marker. At various time points following inoculation of the co-culture, cells are plated on selective media to see whether the aged cells outcompete the un-aged cells, a hallmark of the GASP phenotype. Results illustrating this can be seen in Figure 2.

Descriptions of the three other components of the module are as follows.

Examination of RpoS activity – The purpose of this experiment is to highlight the difference between genotypic and non-genotypic (physiological) changes that occur in a cell to survive a stressful environment. Students assay for the activity of RpoS, a sigma factor typically downregulated in long-term stationary phase, at various stages of culture growth by pipetting a small volume of liquid culture onto a microscope slide and adding a drop of hydrogen peroxide. This allows for the detection of catalase, an enzyme upregulated by RpoS.

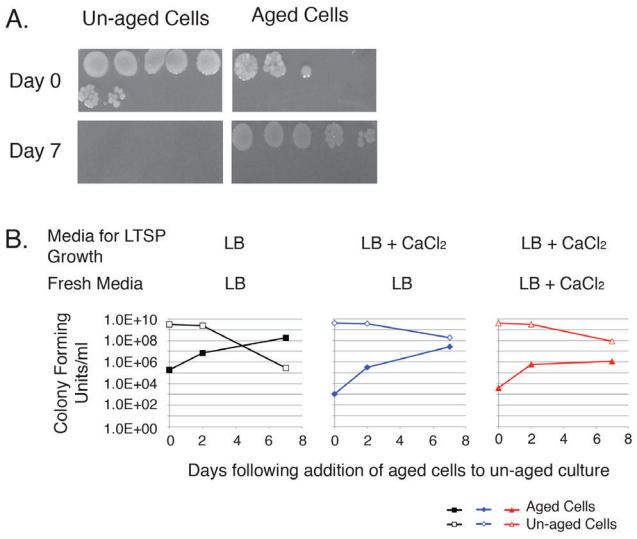

FIGURE 2. Examples of student data illustrating the GASP phenotype. (A) Dilution plating on selective media. Cells are from the co-culture experiment (started in week three of the module). Day 0 refers to the initial culture inoculation and day 7 refers to one week of co-culture growth. Aged cells are distinguished from un-aged cells by the presence of distinct antibiotic resistance markers present in each strain. Cultures are plated with ten-fold serial dilutions. (B) Colony forming units (CFU) per ml of culture are calculated from the dilution plates and graphed on a plot of CFU/ml versus time. The GASP phenotype is illustrated by greater survival values of aged cells over time compared to the un-aged population in the culture. Growth in different types of media can impact the GASP phenotype.

Cell stress measurement – Students can determine whether different environments lead to changes in stress levels in the cells, and whether this correlates with GASP activity. The *E. coli* utilized in the experiment express a stress activated lacZ reporter gene. Students extract cells grown in the different types of media, lyse them, and add a β-galactosidase substrate, the product of which is then detected by a spectrophotometer.

Examination of mutation frequency – Students determine whether mutation frequency of the cells differs between the two growth conditions, and how this relates to cell stress and the GASP phenotype. *E. coli* grown in different media are plated on both LB and LB plus rifampicin. The strain is rifampicin sensitive, but it is possible for spontaneous mutations to arise that allow for resistance. Students calculate the frequency of these spontaneous mutations and compare this value between strains grown in the two environments.

Students complete one post-module assignment in the form of a worksheet (Appendix 4). One part is to provide the experimental hypothesis, which is supported by the literature. The remainder of the worksheet collects the module data, and asks the students to draw conclusions regarding the relationship between the GASP phenotype, genotypic or phenotypic changes in the cell, cell stress, and mutation frequency. Students also update their raw data into an online form to allow for later analysis of the class's data by the authors.

Faculty instructions

In lecture of week one of the module, the instructor spends roughly an hour introducing the GASP phenotype and outlining the experimental protocol used to determine whether it is occurring. This also includes an introduction to dilution plating and how to calculate culture density. The other module components, while not being conducted during week one, are also briefly introduced. The slides used in this lecture are included in the supplemental materials (Appendix 3). During week two of the module, more time is spent introducing the other experimental protocols along with data analysis problems to get students acclimated to the work they will be conducting in the coming weeks. Lectures in weeks three and four reinforce these ideas with further practice problems.

While the individual protocols are fairly straightforward, they do require a tutorial on proper pipetting and sterile technique. Students are provided this in a hands-on manner in the lab sections. The biggest challenge is whether

the students can multi-task, as the various components of the module are performed at once, and not sequentially (Fig. 1). This demand can be lessened by frequent reminders regarding what is occurring in lab each week and by focusing on the purpose of each experimental step, to strengthen students' understanding of why each is necessary.

A logistical issue involved with the experiment is the number of cultures that need to be maintained. For a lab with 100 students (50 pairs), 100 liquid cultures are inoculated during week one. These are small cultures (5 mL) and can be maintained in tubes as opposed to flasks, but must be grown in a shaking incubator set at 37°C for the next two weeks. During week three, each pair of students sets up three co-culture tubes (150 total) along with two tubes for the RpoS assay (an additional 100 tubes). The co-culture tubes are grown for the next week, while the RpoS tubes are only maintained for two days.

There were no other major issues with the experiment in the two quarters where it was incorporated into the curriculum. As this is novel research, there is no required result for the students to obtain. Thus, any data that students believe is "wrong" can actually be used as an exercise for them to generate a plausible explanation based on their background knowledge and the scientific literature. As real research, the goal is also to analyze the student data following module completion. If this is something you are interested in being involved with, please contact the corresponding authors.

Suggestions for determining student learning

Assessment of the student learning outcomes can be completed with a variety of methods, including pre-/post-testing (Fig. 3A, Appendix 5), self-assessment questions (Fig. 3B, Appendix 6), course exam questions (Fig. 3D, Appendix 6), a pipetting test (Fig. 3C, Appendix 7) and the worksheet to be completed at the end of the module (Appendix 4). The pre-/post-test consists of 12 content and data analysis questions that cover a number of the module learning objectives. It is important that both pre- and post-test are administered to students under the same testing conditions to obtain accurate results. For the self-assessment, students are presented with two statements and asked to rate their level of agreement. Course exams as well as the worksheet can be used to measure student learning, although not in a pre/post fashion, making it more difficult to ascertain the specific impact of the module. The worksheet allows students to submit their hypothesis and conclude based on their data whether the evidence supports it. The students are instructed that the hypothesis should be testable, based on prior research or knowledge, contain the independent and dependent variables from the experiment, and include a prediction. The supporting evidence should be from one or more primary research articles. Finally, a pipetting test was utilized both before and after completion of the module to assess pipetting skill as well as the ability to perform dilution calculations. Students are randomly assigned a dilution to perform, and within three minutes must dilute bromophenol blue dye in water. The accuracy of this dilution is determined by spectrophotometer, with the resulting optical density value compared to that of a standard dilution generated by the instructor.

Sample data

Students collected a variety of data to characterize the GASP phenotype. Evidence of GASP by the cells is determined by collecting cell viability numbers at various days in co-culture growth for the aged and un-aged cells (Fig. 2). Students also collect cell stress data, measured as expression of the β-galactosidase reporter relative to the number of viable cells; data on RpoS activity, based on whether or not the enzyme catalase is expressed over the course of the experiment; and cellular mutation frequency values, which are the fraction of cells in culture that are spontaneously resistant to the antibiotic rifampicin.

Safety issues

Students should be instructed to perform standard cleanup procedures for work with microbes along with the use of proper sterile technique. This includes wiping down lab benches with bleach before and after class; wearing gloves, a lab coat, long pants, and close-toed shoes; and disposing of contaminated items in the proper biohazard containers. While we utilized Bunsen burners for this experiment, the Education Board of the American Society for Microbiology (ASM) released a series of guidelines for microbiology teaching laboratories, one of which suggested the use of incinerators or disposable transfer devices instead (3). The *E. coli* strains also contain antibiotic resistance genes (inserted into the genome), and are thus classified as BSL-2 organisms. ASM recommends the use of face shields or goggles when working with these organisms, in addition to the above-mentioned personal protection requirements. These organisms also must be disposed of in proper biohazard containers and the area in which they are utilized must be secured. Please refer to the ASM recommendations for further information (3). All other reagents utilized in the experiment are non-hazardous.

Assessment of the module and dissemination of the data was performed in accordance with UC Irvine Institutional Review Board (IRB) approval (application #2012-9025).

DISCUSSION

Field testing

The UC Irvine Microbiology lab course consists of a two-hour lecture attended by all students in the course (100 students each in Fall 2014 and Winter 2015) followed by four hours of lab split over two days, a three hour "lab"

one day and a one hour "discussion" two days later. The course instructor leads the lecture, designs the curriculum, supervises the graduate student teaching assistants and develops the course assessments. The graduate student–led lab sections consist of 20 students (5 sections per quarter).

The GASP module is just one of the activities encountered by the students during the course. Other parts of the curriculum include the isolation and identification of bacteria, examination of antibiotic resistance in the isolated bacteria, characterization of worm capture by a nematophagous fungus (10), and a brewing module (11). The GASP module was scheduled for the last half of the course, and was performed simultaneously with the fungus and brewing experiments. Students in both quarters achieved the learning objectives, as described below, and based on anecdotal evidence seemed to enjoy the module, particularly the fact that they were engaging in "real" research.

Evidence of student learning

The module learning objectives and the means of assessment are listed in Table 1. Assessment occurred through a pre-/post-test, student self-assessment, pipetting test, course exams, and a worksheet completed at the end of the module. The pre-/post-test included questions focused on module-related concepts, including bacterial growth and evolution, as well as the ability to perform dilution calculations, read a micropipettor, and write a hypothesis (Appendix 5). The tests were administered in the lab sections a week before the module began (pre) and the same week as module completion (post). Students were allowed 15 minutes for each test and were awarded one point of extra credit for completion. The questions or answers were not discussed at any time during the course. As evident from Figure 3A, student performance for each question was significantly higher following completion of the module ($p < 0.001$). The one exception to this was question 4, which was a dilution calculation. Based on the fairly high percentage of students who correctly answered this question the first time, it may be that the students were sufficiently capable of this prior to the module. In addition, positive gains were seen in the pipetting test (Fig. 3C), which also required a dilution calculation. Despite the statistically significant gains, students performed poorly on questions regarding the role of salts in the growth medium and reporter genes (questions 2 and 3). The former is not necessarily surprising, as there were multiple ways for students to modify the LB, including salts, amino acids, and sugars. Thus, students that did not select a salt as their added ingredient likely would not research its potential impact. The poor performance on the reporter gene question was surprising, as a molecular biology course is a prerequisite for the microbiology lab. In future iterations, we will include more background information on reporter genes in the course lecture.

Students were also asked to self-assess their ability to use micropipettors and discuss the molecular basis of evolution. They were presented with a statement related to each and asked to rate their level of agreement on a 5-point Likert scale (5 = strongly agree to 1 = strongly disagree) using the iClicker personal response system during lecture. For both quarters assessed, we presented the students with the statements at the end of the module, asking them to measure their current abilities as well as what they believe they were capable of prior to the start of the module. This was done due to minimize response-shift bias, which describes the fact that individuals may inaccurately self-assess prior to an activity due to a lack of context regarding that task (6). For both questions, students felt that they had significantly improved following completion of the module (Fig. 3B). During the second quarter the module was performed, we also presented these statements prior to beginning the experiment in true pre-assessment fashion. There was also a statistically significant gain between this pre-assessment and the post self-assessment. In addition, these pre rankings were higher than the retrospective self-assessment conducted at the end of the quarter (data not shown).

At the beginning of the module, students were instructed on the proper usage of micropipettors and were provided instructions on how to perform a serial dilution. Following completion of this exercise, each student was given a three-minute pipetting test, in which they selected a random dilution of a dye to perform, the accuracy of which was measured by a spectrophotometer. This test was also implemented following module completion. As seen in Figure 3C, the fraction of the class passing the test was significantly higher post-module.

A final means of assessment was performance on module-related questions on course exams. Students were presented with questions on evolution, analysis of GASP data, and the experimental protocols, among others (Appendix 6). In addition, exams included material from all other topics covered during the course. To compare performance between module-specific questions and questions covering other topics that have been previously assessed (10, 11), some of which have been used for a considerable amount of time in the course and are much more established, we separated questions into lower Bloom's levels (Bloom's 1–2) and higher Bloom's levels (Bloom's 3–6). Bloom's taxonomy is used as a means to characterize different types of thinking required to answer a given question (2, 7). As GASP-specific questions only covered Bloom's 2–5, we examined performance on Bloom's level 2 questions and Bloom's level 3, 4, and 5 questions. As seen in Figure 2D, student performance on Bloom's level 2 module-specific questions did not differ from other Bloom's level 2 questions, although performance on the higher Bloom's level questions was significantly lower. We speculate that this may be due to differences in exam question difficulty or because the GASP topic in general is more difficult to comprehend compared to other material. Results on the test questions also emphasize that in future iterations we need to focus more on certain topics, such as reporter

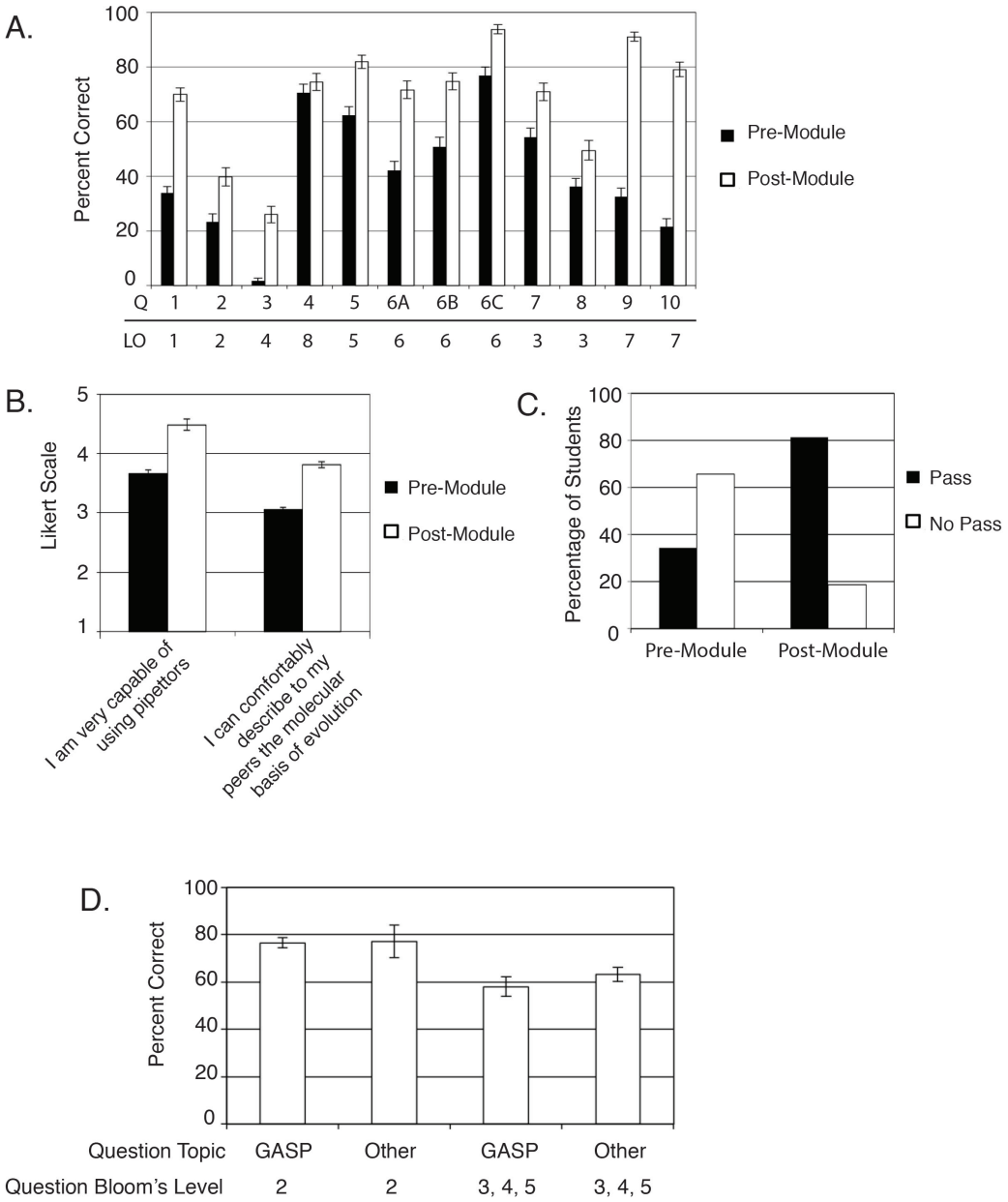

FIGURE 3. Assessments confirm that students achieved the module learning objectives. (A) A 12-question pre-/post-test was administered before and after the GASP module in laboratory sections during Fall quarter 2014 and Winter quarter 2015 (n = 197 students combined). Performance on each question (Q) and the corresponding learning objective (LO) it assessed is indicated. All post-test gains are statistically significant ($p < 0.001$ by t-test) with the exception of question 4. (B) Students noted their agreement with the indicated statements on a 5-point Likert scale (5 = strongly agree, 1 = strongly disagree) upon completion of the module. They were asked to state their current agreement and provide a retroactive agreement (the pre-module data). Post-test gains are statistically significant ($p < 0.001$ by chi-square test). Questions were asked using the iClicker response system (n = 190 students). (C) Student performance on the pipetting test before and after module completion (n = 198 students). Passing refers to whether a student's dilution fell within 0.050 OD_{595} units from a standard value obtained by the course instructor who performed the same dilutions multiple times. The number of students who passed post-module was significantly greater than pre-module ($p < 0.001$ by t-test). (D) Student performance on exam questions categorized by whether or not they were GASP module-specific and by Bloom's level (Bloom's 2 versus 3, 4, and 5) (n = 201 students). The difference between GASP and Other questions of Bloom's level 3, 4, and 5 was statistically significant ($p < 0.001$ by t-test). Question numbers in each category were GASP (BL2) = 5, Other (BL2) = 10, GASP (BL3, 4, 5) = 19, Other (BL3, 4, 5) = 38.

genes and analysis of the Luria and Delbrück experimental data. While we make no claims that this analysis of the experimental data demonstrates that students "sufficiently" learned the material, it does provide a learning baseline by examining it in the context of performance on questions from more established experiments.

Possible modifications

The various portions of this experiment are distinct in nature, and thus depending on the available time in the curriculum and the abilities of the students, it is possible to choose which are appropriate for one's course. On its own, the GASP co-culture experiment is discovery-driven, and enables students to choose a variable, develop an evidence-based hypothesis, and collect and analyze novel data. While the other three components of the module allow for further characterization of the phenotype, and demonstrate to students how evolution is guided by a multitude of factors, they can be removed at the discretion of the course instructor. For example, introductory students or nonmajors may have trouble with the concept of using a reporter gene to measure cell stress, and thus the β-galactosidase assay could be eliminated. Scheduling issues can also be accommodated by the flexible nature of the experiments. For example, if a lab course only meets once a week, much of the work that we performed in the second lab period can actually be performed the following week.

We would also recommend expanding the worksheet currently used into a complete laboratory report formatted like a scientific paper. Our crowded lab schedule would not allow for this addition, but a report would have a number of benefits, such as highlighting the fact that students are conducting original work and forcing them to think more about their data, in terms of how to present it and how their conclusions relate to the scientific literature.

SUPPLEMENTAL MATERIALS

Appendix 1: Reagent/equipment list and faculty instructions
Appendix 2: Student handout and protocol
Appendix 3: Lecture slides
Appendix 4: GASP worksheet
Appendix 5: Module pre-/post-test
Appendix 6: Self-assessment and exam questions
Appendix 7: Pipetting test instructions
Appendix 8: Example student hypotheses

ACKNOWLEDGMENTS

We would like to thank B. Almourzaeva and O. Shimizu for assistance with experiment preparation, and S. E. Finkel for helpful discussion. The authors declare that there are no conflicts of interest.

REFERENCES

1. **Alters, B. J., C. E. Nelson, and J. Mitton.** 2002. Perspective: teaching evolution in higher education. Evolution **56**:1891–1901.
2. **Crowe, A., C. Dirks, and M. P. Wenderoth.** 2008. Biology in bloom: implementing Bloom's taxonomy to enhance student learning in biology. CBE Life Sci. Educ. **7**:368–381.
3. **Emmert, E. A. B., and ASM Task Committee on Laboratory Biosafety.** 2013. Biosafety guidelines for handling microorganisms in the teaching laboratory: development and rationale. J. Microbiol. Biol. Educ. **14**:78–83.
4. **Farrell, M. J., and S. E. Finkel.** 2003. The growth advantage in stationary-phase phenotype conferred by rpos mutations is dependent on the ph and nutrient environment. J. Bacteriol. **185**:7044–7052.
5. **Finkel, S. E.** 2006. Long-term survival during stationary phase: evolution and the GASP phenotype. Nat. Rev. Microbiol. **4**:113–120.
6. **Howard, G. S.** 1980. Response-shift bias: a problem in evaluating interventions with pre/post self-reports. Eval. Rev. **4**:93–106.
7. **Krathwohl, D. R.** 2002. A revision of Bloom's taxonomy: an overview. Theory Pract. **41**:212–218.
8. **Luria, S. E., and M. Delbrück.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**:491–511.
9. **Miller, J. D., E. C. Scott, and S. Okamoto.** 2006. Public acceptance of evolution. Science **313**:765–766.
10. **Sato, B. K.** 2013. Attack of the killer fungus: a hypothesis-driven lab module. J. Microbiol. Biol. Educ. **14**:230–237.
11. **Sato, B. K., U. Alam, S. J. Dacanay, A. K. Lee, and J. F. Shaffer.** 2015. Brewing for students: an inquiry-based microbiology lab. J. Microbiol. Biol. Educ. **16**(2):223–229.
12. **Smith, M.** 2010. Current status of research in teaching and learning evolution: II. pedagogical issues. Sci. Educ. **19**:539–571.