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Antibiotic resistant pathogen outbreak investigation: an interdisciplinary module to teach fundamentals of evolutionary biology

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Abstract

The evolution of resistance to antibiotics provides a timely and relevant topic for teaching undergraduate students evolutionary biology. Here, we present a module incorporating modified sequencing data from eight antibiotic resistant pathogen outbreaks in hospital settings with bioinformatics and phylogenetic analyses. This module uses whole genome sequencing data from hospital outbreaks investigated by the Centers for Disease Control and Prevention to provide examples of antibiotic resistance spread. Students work in groups to analyze outbreak data to identify the bacterial species and antibiotic resistance genes, to infer a phylogenetic tree examining relatedness among isolates, and to determine a possible source of the outbreak. Students then compile their results in individual reports and provide recommendations for preventing the further spread of antibiotic resistant organisms. In addition to providing genomic outbreak data, we include a teaching concepts guide discussing three integral components of the module: how evolutionary biology concepts of natural selection and competition impact antibiotic resistance; outbreak investigation information to aid in phylogenetic analysis and creation of recommendations; and instructions for the bioinformatics protocol. Completion of this module provides students an opportunity to think critically about the evolution of resistance, practice bioinformatics techniques, and relate evolutionary biology to current events.

Keywords

Evolution; Bioinformatics; Phylogenetics; Integrative Learning; Data Sets

Background

For students to conceptualize evolutionary biology and its importance, we must emphasize its relevance in everyday life with pertinent examples (Hillis 2007, Chamany et al. 2008,

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Data Sets

Data sets and materials are available at <http://pierceresearch.weebly.com/teaching-materials.html>

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Thanukos 2009). News and journalism reports center daily on topics driven by evolutionary biology, such as antibiotic resistance, genetically modified organisms, invasive species, and zoonotic diseases (Desantis 2009), providing us numerous examples for the classroom. Additionally, there have been calls for collaboration between evolutionary biologists and medical researchers to develop curricula that integrate evolutionary biology with public health (Antolin et al. 2012) as there is a misconception that these fields have little overlap (Antonovics et al. 2007, Nesse and Stearns 2008). By using relevant examples in the classroom, students will be better able to transfer their knowledge from the textbook to daily life. Aside from increased understanding, the use of these examples allows instructors to integrate a number of biological concepts and prepare students to deal with the interdisciplinary nature of most “real-world” problems (Chamany et al. 2008).

One way to integrate other disciplines with evolutionary biology is through the use of bioinformatics analyses, which incorporates genetics, computer science, and other fields depending on the data set. Introduction to bioinformatics is important for general biology majors as bioinformatics tools are becoming increasingly common in research (Perkel 2017). Increased recognition of the need to introduce bioinformatics to students (Pevzner and Shamir 2009, Ditty et al. 2010) as well as to better integrate molecular genetics perspectives in evolutionary biology (Smith et al. 2009) creates an opportunity for novel classroom activities. Here, we present a module providing students an opportunity to apply principles of evolutionary biology to a “real life” example by integrating bioinformatics techniques with modified genetic data sets from antibiotic resistant pathogen hospital outbreaks.

The Module

Antibiotic resistance has been identified as a modern day example of evolution in action and ripe for use in the classroom (Cloud-Hansen et al. 2008, Bohlin and Host 2015). Not only can it occur rapidly, allowing students to see and conceptualize evolution, but it is also considered a major public health threat. Every year, over 2 million people become infected with antibiotic resistant organisms resulting in an estimated 23,000 deaths in the United States alone (CDC 2013).

Antibiotic resistance encompasses evolutionary topics like natural selection, variation, and competition (Figure 1). For example, understanding population variation due to random mutation is important in understanding how natural selection is able to drive resistance. Additionally, knowledge of intra- and inter-species competition can help students understand how antibiotic use impacts the rate of carbapenem-resistant Enterobacteriaceae (CRE) resistance or the emergence of diseases like *C. difficile* infection.

Also relevant and critical to evolutionary biology is the use of phylogenetic trees which are common tools in outbreak analyses to investigate the spread of disease (Didelot et al. 2012, Sintchenko and Holmes 2015). This module deals with an antibiotic resistant pathogen outbreak, providing a hands-on, integrative learning activity where students combine knowledge of evolutionary biology, basic microbiology, and bioinformatics. This module allows students an opportunity to use bioinformatics and phylogenetic analyses to “solve the

outbreak” and is well suited for an evolutionary biology course or a more specialized course, like disease ecology or evolutionary medicine.

A pilot version of the module was implemented at the University of North Carolina at Pembroke, which is a public, historically American Indian liberal arts university in rural North Carolina. We created this module for a course titled “Ecology and Evolution of Disease”, which focused on understanding the importance of ecological and evolutionary principles in the context of disease and human health. This was a new course at the university developed and taught by the first author. The class was an upper level special topics course composed of 20 undergraduate biology majors- mainly juniors and seniors. After the pilot module was completed, the bioinformatics protocol was updated to address common mistakes or areas of confusion for students.

Data sets and materials

We present eight independent data sets from hospital outbreaks which occurred throughout the United States. These data sets were originally analyzed by the Centers for Disease Control and Prevention’s national laboratory for clinical and environmental microbiology, which tests bacteria isolates from around the US to detect new and emerging resistance patterns that affect patient health. These data sets include bacterial isolates from patients and the hospital environment, enabling students to determine a possible source and spread of the outbreak. We also included unrelated isolates to clonal data sets to make them suitable for simple phylogenetic analysis. Each outbreak data set includes two files: a representative genome assembly and a Single Nucleotide Polymorphism (SNP) position file. The representative genome assembly is provided to allow students to determine the bacterial species responsible for the outbreak and identify genes conferring antibiotic resistance. The SNP position file details SNPs from all bacterial isolates which students use to create a phylogenetic tree to visualize the outbreak and identify a likely route of transmission. In addition to the data sets, we also include the publically available metadata which provides information detailing the origin of the samples (e.g., patient, vacuum cleaner, sink), as well as the location and type of hospital where the outbreak occurred.

We also make available a teaching concepts handout with resources for teaching natural selection, competition, and phylogenetics (specific to outbreaks) in an antibiotic resistance framework. Finally, we provide PowerPoint slides covering topics like: signs of a hospital outbreak, ways to detect and prevent outbreaks, and a brief introduction to whole genome sequencing and genomics. When we implemented the module, A.A. Pierce led lectures covering evolutionary biology concepts and antibiotic resistance, and T.J.B. de Man lectured on hospital outbreak examples and genomic analyses. As course instructor, A.A. Pierce was present while students worked through the module protocol and presented their results.

Methods for module

Antibiotic resistance allows instructors to cover topics such as natural selection, variation, competition, and phylogenetics. An in depth discussion of these topics should be covered first in the context of antibiotic resistance for students to gain the most of the module. To

begin the module, we divided 20 students into eight groups composed of 2–3 students. Each group was assigned one of the eight outbreaks and access to the associated metadata, bioinformatics protocol, and a summary report grading rubric.

This protocol requires use of bioinformatics software including SSTAR v1.1.01 (de Man and Limbago 2016), BLAST v2.6.0 (Altschul et al. 1990) and MEGA6 (Tamura et al. 2013), which need to be installed locally prior to the module. In addition, web-based tools RNAmmer (Lagesen et al. 2007) and iTOL (Letunic and Bork 2016) are also used and require an internet connection.

To begin the module, students use the representative genome assembly file to identify the bacterial species responsible for the outbreak (Figure 2). To do this, students use the representative genome assembly file as input for RNAmmer (<http://www.cbs.dtu.dk/services/RNAmmer/>) to identify a 16S rRNA gene. Students then use the predicted 16S gene sequence as a query for web-based NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the bacterial species. Once students have determined this, they now use the representative genome assembly file as input for the SSTAR program, which identifies known antimicrobial resistance genes. With this output, depending on the focus of the course, students can further explore these genes and determine which antibiotics the bacteria are likely resistant to and which resistance mechanisms (e.g. efflux pump, enzyme, outer membrane porin) are possibly conferring resistance.

The next step in the module is to determine a likely source of the antibiotic resistant pathogen and track its transmission (Figure 2). To do so, students use the outbreak SNP position file with the program MEGA6 to create a maximum likelihood phylogenetic tree. As outbreaks happen rapidly, there is little time for many new genetic mutations to accumulate and students can therefore identify an outbreak using a phylogenetic tree based on highly related samples. In ideal cases this is shown by samples connected by a (near) vertical branch (Figure 3). To further analyze the outbreak, students are encouraged to edit their phylogenetic tree in the web-based program iTOL (<https://itol.embl.de>), where they are able to color code isolate labels and branches based on the metadata file provided.

Once completed, the students have finalized the bioinformatics portion of the module and have successfully analyzed an antibiotic resistant pathogen outbreak in a manner similar to the “Disease Detectives” at the Centers for Disease Control and Prevention.

Student synthesis

If implemented at other institutions, instructors are encouraged to ask students to synthesize this material in the form of a report or a presentation (Figure 4). During our module implementation, students were encouraged to work with their group during the outbreak investigation but write independent reports which accounted for the majority of the grading (students were also graded on a group oral presentation and group peer-evaluations). Students were instructed to write their report as if they were investigators at the Centers for Disease Control and Prevention and needed to summarize their findings for the hospital or

state health department. Along with data sets and materials, we also provide a sample of a report rubric as guidance for instructors.

Creation of a presentation or report compels students to think critically about their phylogenetic analyses by pinpointing the samples involved in the outbreak, as well as a likely source of transmission, and relating their findings to a “real-world” situation. For example, by determining the antibiotic resistance genes, students identified the likely mechanism of resistance (e.g. mutated efflux pump) and made suggestions on types of antibiotics that may not be effective for treatment. It also promotes communication skills and practice presenting scientific findings to an audience. Additional questions that may be asked of students are: (1) What recommendations would you make to the hospital to prevent future outbreaks? (2) How would additional sampling potentially alter your conclusions? (3) In what ways do you think aspects of the hospital (e.g. department/unit, type of procedures) impacted the outbreak?.

Conclusions

Despite the relevance of evolutionary biology to many of today’s headlines, there is still an ongoing need to demonstrate its importance, including to undergraduate students (Hillis 2007, Thanukos 2009). We believe that this module can be successfully implemented by instructors at other institutions to work towards this need. Using antibiotic resistant pathogen outbreaks as an example, students can see how topics like natural selection and competition can aid in understanding an urgent public health threat as well as gain hands on experience in bioinformatics and phylogenetic techniques. Inquiry-based activities that actively engage students are powerful tools in solidifying concepts (Allen et al. 1996) and “solving the outbreak” can facilitate the learning of evolutionary biology fundamentals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES CITED

- Allen DE, Duch BJ, and Groh SD. 1996 The power of problem-based learning in teaching introductory science courses. *New Directions for Teaching & Learning* 1996:43–52.
- Altschul SF, Gish W, Miller W, Myers EW, and Lipman DJ. 1990 Basic local alignment search tool. *Journal of Molecular Biology* 215:403–410. [PubMed: 2231712]
- Antolin MF, Jenkins KP, Bergstrom CT, Crespi BJ, De S, Hancock A, Hanley KA, Meagher TR, Moreno-Estrada A, Nesse RM, Omenn GS, and Stearns SC. 2012 Evolution and medicine in undergraduate education: a prescription for all biology students. *Evolution* 66:1991–2006. [PubMed: 22671563]

- Antonovics J, Abbate JL, Baker CH, Daley D, Hood ME, Jenkins CE, Johnson LJ, Murray JJ, Panjeti V, Rudolf VHW, Sloan D, and Vondrasek J. 2007 Evolution by any other name: Antibiotic resistance and avoidance of the E-word. *Plos Biology* 5:137–140.
- Bohlin G, and Host GE. 2015 Evolutionary explanations for antibiotic resistance in daily press, online websites and biology textbooks in Sweden. *International Journal of Science Education Part B-Communication and Public Engagement* 5:319–338.
- CDC. 2013 Antibiotic resistance threats in the United States, 2013. [www.cdc.gov](http://www.cdc.gov/drugresistance/threat-report-2013) Available at: <http://www.cdc.gov/drugresistance/threat-report-2013>. Available at: <http://www.cdc.gov/drugresistance/threat-report-2013>.
- Chamany K, Allen D, and Tanner K. 2008 Making biology learning relevant to students: integrating people, history, and context into college biology teaching. *Cbe-Life Sciences Education* 7:267–278. [PubMed: 18765745]
- Cloud-Hansen KA, Kuehner JN, Tong LL, Miller S, and Handelsman J. 2008 Money, Sex, and Drugs: A Case Study to Teach the Genetics of Antibiotic Resistance. *Cbe-Life Sciences Education* 7:302–309. [PubMed: 18765752]
- de Man TJB, and Limbago BM. 2016 SSTAR, a stand-alone easy-to-use antimicrobial resistance gene predictor. *Msphere* 1.
- Desantis LRG 2009 Teaching evolution through inquiry-based lessons of uncontroversial science. *The American Biology Teacher* 71:106–111.
- Didelot X, Bowden R, Wilson DJ, Peto TEA, and Crook DW. 2012 Transforming clinical microbiology with bacterial genome sequencing. *Nature Reviews Genetics* 13:601–612.
- Ditty JL, Kvaal CA, Goodner B, Freyermuth SK, Bailey C, Britton RA, Gordon SG, Heinhorst S, Reed K, Xu ZH, Sanders-Lorenz ER, Axen S, Kim E, Johns M, Scott K, and Kerfeld CA. 2010 Incorporating Genomics and Bioinformatics across the Life Sciences Curriculum. *Plos Biology* 8.
- Hillis DM 2007 Making evolution relevant and exciting to biology students. *Evolution* 61:1261–1264. [PubMed: 17542837]
- Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, and Ussery DW. 2007 RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Research* 35:3100–3108. [PubMed: 17452365]
- Letunic I, and Bork P. 2016 Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research* 44:W242–W245. [PubMed: 27095192]
- Nesse RM, and Stearns SC. 2008 The great opportunity: Evolutionary applications to medicine and public health. *Evolutionary Applications* 1:28–48. [PubMed: 25567489]
- Perkel JM 2017 How bioinformatics tools are bringing genetic analysis to the masses. *Nature* 543:137–138. [PubMed: 28252089]
- Pevzner P, and Shamir R. 2009 Computing Has Changed Biology-Biology Education Must Catch Up. *Science* 325:541–542. [PubMed: 19644094]
- Sintchenko V., and Holmes EC. 2015 The role of pathogen genomics in assessing disease transmission. *Bmj-British Medical Journal* 350.
- Smith JJ, Baum DA, and Moore A. 2009 The need for molecular genetic perspectives in evolutionary education (and vice versa). *Trends in Genetics* 25:427–429. [PubMed: 19801171]
- Tamura K, Stecher G, Peterson D, Filipinski A, and Kumar S. 2013 MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30:2725–2729. [PubMed: 24132122]
- Thanukos A 2009 From newsroom to classroom. *Evolution: Education and Outreach* 2.

Figure 1. Teaching concepts relevant to module

. Antibiotic resistance provides students with an example of evolution in action and covers evolutionary topics like natural selection, competition, and phylogenetics.

BIOINFORMATICS PIPELINE:

Figure 2. Module protocol schematic

. The outbreak investigation model introduces students to basic bioinformatics techniques. Students are able to identify the species of bacteria and genes conferring resistance using a representative genome assembly from the outbreak data set. Students also use a SNP position file to infer a phylogeny of outbreak isolates.

Figure 3. Example of a student-made phylogeny

. Above is a final product from one of the outbreaks which was created in MEGA6 and edited using iTOL. The isolate types were color coded for easy interpretation. The student was able to identify the samples involved in the outbreak by the nearly vertical branch patterns connecting the patients and catheter samples. This branching pattern is indicative of low genetic differentiation as expected among related isolates in an outbreak.

Figure 4. Student synthesis of module

. After module completion, instructors are encouraged to ask students to present their findings in either an oral presentation or a written report. Findings can include the correct identification of the bacterial species and resistance genes, a description of the outbreak spread, and recommendations for the hospital. These findings are unique to the outbreak and require students to think critically about their results.