INQUIRY & INVESTIGATION

CUREs: How to Create & Incorporate a Collaborative Ant-Based Project to Teach Science Practices

Carrie Jo Bucklin, Laurie Mauger

Abstract

Course-based undergraduate research experiences (CUREs) are one way instructors can increase engagement and learning of material. One of the goals in the report Vision and Change in Undergraduate Biology Education: A Call to Action *is to increase active learning activities. By implementing a CURE or CURE-type model, instructors provide students with the opportunity to develop a better understanding of science content, to apply what they have learned, and make an impact in real-world science. Our classes replicated a subset of the work being completed in Gorongosa National Park in Mozambique. We had students complete biodiversity surveys through collection and classification of ant species using field and lab techniques. DNA barcoding analyses are commonly used techniques in biology labs worldwide. Polymerase chain reaction and cycle sequencing will be taught to illustrate how the extracted DNA can be amplified at different markers and used to identify species. We utilized the CURE model to have students complete a biodiversity survey of both a southern intermountain-west and a southeastern state through collection, classification, and genotyping and barcoding of ant species.*

Key Words: *course-based undergraduate research experience; genetics; population genetics; ecology.*

○ Introduction

There has been extensive research investigating how course-based undergraduate research experiences (CUREs) help students develop a better understanding of science content (American Association for the Advancement of Science, 2010; Blanton, 2008; Frantz et al., 2006; Hunter et al., 2007; Stein et al., 2004). Utilizing CUREs rather than the traditional apprenticeship model allows students to learn about existing research opportunities and the benefits of partaking in them, and it removes the perceived cultural norms and barriers to interacting with faculty (Bangera & Brownell, 2014). By removing these barriers, we can encourage more students to become involved in scientific research. Bangera and Brownell (2014) have determined that CUREs can decrease the barriers to research that are felt by minoritized (URM) students.

Our classes replicated a subset of the work being completed in Gorongosa National Park (GNP) in Mozambique. Researchers in GNP are investigating how biodiversity of an area increases after it has been destroyed by human impact. One of the GNP projects utilized ants as a measure of ecosystem health. The diversity of ant species may be an indicator of local ecosystem health and changes (Bestelmeyer, 2005; Bestelmeyer & Wiens, 2001; Folgarait, 1998; Nash et al., 2001). We replicated the collection and identification (to the genus level) of ant species from a GNP project at a primarily undergraduate institution (PUI) and a research intensive (R1) institution. Both groups of students included URM students. By developing these courses as CUREs, the barriers to research typically experienced by students (e.g., time and funding for projects) were reduced; students were given dedicated class time to complete the projects, and course funding was used to purchase supplies. Replicating a previously designed project from a different environmental location was done for two reasons:

- Students who have never done research before can benefit from additional scaffolding. They watched videos of researchers from GNP as well as reading research papers to understand how the ant collections would work. This process mimics what is done in many mentored research groups.
- Educators can also benefit from project scaffolding. They do not always have time to create a brand new project, and utilizing a previously designed method in a new location is a faster way for educators to incorporate CUREs.

Students from both the PUI and R1 institution were exposed to work being completed by individuals who break stereotype barriers (many of the researchers at GNP are underrepresented in the sciences) to illustrate to our students that science is for all people and that anyone can do science. All students collected ants, identified to the genus level using dichotomous keys, and estimated biodiversity of their respective areas by calculating species richness, species abundance, Shannon diversity index, and evenness.

Students at the PUI were enrolled in an environmental biology (EnvBio) and a genetics (Gen) course. EnvBio is a small-enrollment course (24 students per semester) intended for first- or second-year

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nonbiology majors. The course is a lecture-only course, but **we** designed it to be laboratory heavy (i.e., a field course) so students could see environmental research in action to get a better understanding of environmental biology. Students enrolled in EnvBio focused on ant collection/identification, data analysis, and presentation skills. These students were asked to think about how their experiences on the project could be used in their future careers. These students also shared their data and collected specimens with students enrolled in Genetics and Evolution (GenEvo, described ahead) at the R1 to learn about collaboration and comparison of different ecosystems. The Gen course is a sophomore-level course (lecture and lab) required for biology majors and prehealth students at the PUI. This course investigates the transmission and expression of genetic information, organisms, and population genetics. Students enrolled in Gen learned about genotyping at microsatellite markers to investigate genetic diversity in ecosystems with different levels of human impact.

Students at the R1 were enrolled in a GenEvo, a large enrollment (100–400 students per semester) gateway course (lecture and lab) that is required for biology majors and prehealth students and counts as a natural science elective for the R1. This course investigates transmission and population genetics and evolution of organisms and populations. These students learned DNA barcoding, a method of species identification that uses DNA from a specific gene, by amplifying DNA of collected ants at a portion of the cytochrome oxidase I (COI) gene to identify species collected. Students also visually compared DNA and protein sequences to visualize synonymous versus nonsynonymous mutations. These students also utilized specimens sent from the PUI in their analysis to barcode to check for accuracy in the dichotomous key. See Figure 1 for a schematic of course activities and collaborations across institutions.

The place-based activities of the CURE enhanced the drive to do research for some students because they occurred in locations the students were familiar with. A student enrolled in the Gen course (PUI) asked to extend their CURE activities and completed an independent research project investigating the effect of urbanization on ant genetic and species diversity (Garavito et al., 2020). Other students enrolled in the EnvBio and Gen courses (PUI) who participated in the CURE also asked to extend their work from the CURE on a smaller project for additional research experience and practice presenting at small local conferences and regional conferences.

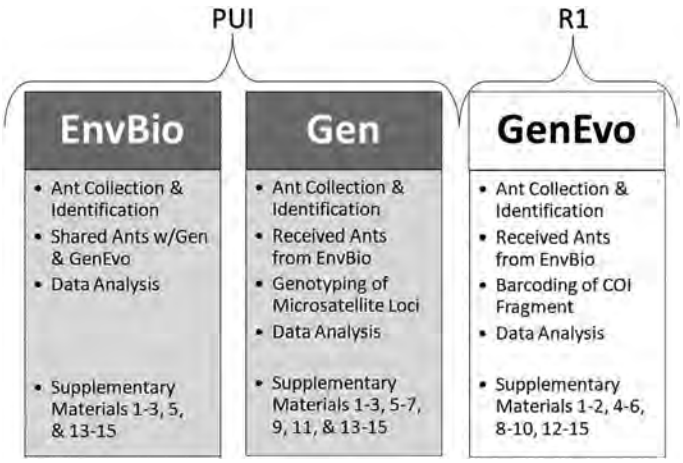

Figure 1. Map of activities by course and collaborative activities across institutions.

Students in the GenEvo course (R1) were also interested in extending research, but funding was not available to support this research. These additional projects illustrate how students can extend CUREs beyond the classroom and gain valuable experiences that benefit future careers.

○ Lesson Guide

We have organized the following section based on the materials we used to run the CURE in both a field course and a lab course with a field component: learning objectives, course schedule, methods (field and genetics), and assessments.

Learning Objectives

The following learning objectives are those we chose as fitting all the courses completing the CURE. Learning objectives can be used as is or modified to suit the needs of the course.

- Engage in scientific practices/discourse.
- Explore how science is a "way of knowing" about the world.
- Explain the components of environmental biology/biodiversity.
- Elaborate on how to build new hypotheses with data collected.
- Evaluate how information gained can be applied to new scenarios.

Course Schedule

We used the following course schedules in a university setting. Table 1 is based on a 16-week (including finals week) schedule whereas Table 2 is a modified schedule. Gen and EnvBio (PUI) followed the schedule in Table 1. Table 2 shows a modified genetics schedule. GenEvo (R1) followed the schedule in Table 2. The design of GenEvo included additional laboratory experiences beyond the CURE, hence the shortened schedule. The rows of each table show the lecture content and the lab (activities) content for each week; our classes meet for two to three hours once per week.

Methods: Field

Ant collection. Students were taught how to collect ants using a collections protocol and a disposable ant aspirator (you can find reusable aspirators, but the disposable ones are easy for students to use and can be made with grocery store items, and the aspirator bags double as specimen containers; Appendix 1). Students collected ants in groups of four (typically six groups total), identifying each week who would be in charge of building the ant aspirators (typically two students, one for each collection site), recording data on the data sheet (Appendix 2), and collecting ants with the aspirator. In EnvBio, students collected data over multiple weeks and were required to rotate jobs each week so each student had the experience and opportunity to collect the ants. In Gen and GenEvo, students collected ants for only one week. Each group had their supplies in a drawstring backpack or plastic container (e.g., aspirator supplies, data sheets printed on waterproof paper, clipboards, GPS units, and pens). We designated a "central base" where there would be extra supplies (paper, batteries, tape, etc.). Each group would walk in a different cardinal direction away from the central base to start their ant hunt. When students found their first ant,

Table 1. Semester schedule for a field-based course and a genetics-based course. This schedule was used by the PUI courses EnvBio, Gen, and R1 (GenEvo).

Lab Week	Field Course (EnvBio) Activities	Genetics Course (Gen) Activities
1	**Lecture:** what is science; why study biodiversity **Activities:** pre-tests; Gorongosa video; practice collecting ants	
2–5	**Lecture:** none **Activities:** ant collection	*Holiday break (2)* **Lecture:** none **Activities:** ant collection (3–5)
6–7	**Lecture:** dichotomous keys; ant characteristics **Activities:** practice dichotomous key (6); ant identification	**Lecture:** dichotomous keys; ant characteristics **Activities:** ant identification
8	**Lecture:** none **Activities:** ant identification	**Lecture:** DNA structure and extraction techniques **Activities**: DNA extraction
9	**Lecture:** statistics **Activities:** data analysis	*Fall break*
10	**Lecture:** weather vs. climate **Activities:** in-class	**Lecture:** DNA amplification **Activities:** PCR
11	**Lecture:** science communication **Activities:** group working day	**Lecture:** gel electrophoresis **Activities:** electrophoresis
12	**Lecture:** ants in an ecosystem **Activities:** poster pre-critique	**Lecture:** population genetics and/or DNA barcoding **Activities:** data analysis
13	*Holiday break*	
14	**Lecture**: anthropogenic effects; revisit science practices **Activities:** poster presentations; peer evaluation forms	
15	**Lecture:** finals week prep **Activities:** post-assessments	

Table 2. DNA barcoding schedule. This schedule was used by the R1 GenEvo course. Students did not work on the ant project for the entire semester.

Lab Week	Genetics Course (GenEvo) Activities
1	**Lecture:** importance of ants in an ecosystem; why study biodiversity **Activities:** Gorongosa video, ant collection
2	**Lecture:** DNA extraction **Activities:** DNA extraction
3	**Lecture:** DNA barcoding, PCR **Activities:** amplification of COI gene (can be combined with Lab 2)
4	**Lecture:** electrophoresis and sequencing methods **Activities:** verification of PCR product, cycle sequencing (we do this for our students but discuss the procedures here)
5	**Lecture:** sequence data analysis, BLAST **Activities:** data analysis (can be split into two labs)

the collector would begin using the aspirator to try to collect the ant(s) within five minutes. We put a time limit on collection as each group needed to collect samples from three shady and three sunny locations (n = 6) and still have time to record data and make it back to the central base in time for their next class. Students collected environmental data (air temperature and humidity, using a weather app on their phone; cloud cover, estimating percentage of coverage in the area directly overhead; any precipitation type, observing visually) at each collection point. When students were finished, they put their labeled specimen containers in a collection bin. The collection bin was stored in a freezer immediately upon return to campus.

Ant identification. After all ant collection was completed for the semester, students were taught how to identify ants to genus using dissecting microscopes, dichotomous keys (Utah—Appendix 3; North Carolina—Appendix 4), and images from entomology texts and Antwiki (https://antwiki.org/wiki/Welcome_to_AntWiki). Instructors described what a dichotomous key is and how to use one. We suggest, if there is time available, that you have students practice with ant images from the web. Students were also taught the basics of how to use dissecting scopes and the differences between those and compound light microscopes. Lastly, students were taught how to use the Ant Identification Data Sheet (Appendix 5) to record the number of each genus found at each location. Students worked in groups of two, with each group taking one specimen bag and

each student identifying each specimen to genus; both students had to agree on the genus for quality assurance. They then counted the number of individuals of each genus and divided the ants into vials based on genus and location ID, giving each vial a unique identifier. The first time identifying ants will take students significantly longer than in subsequent weeks, as their skills and speed increase. We used the Global Ant Biodiversity Informatics (GABI) Project database (Guénard et al., 2017) and antmaps.org (Janicki et al., 2016) to determine whether there were native species for each ant genera identified through our BLAST (Basic Local Alignment Search Tool on the NCBI database) search. Ant vials were stored for later students' genetic analysis (described ahead) or shared with partner institutions for their genetic analysis.

Methods: Genetics

After ant identification was completed, Gen and GenEvo students extracted, amplified, and analyzed ant DNA. Students worked individually or in pairs to extract ant DNA using Qiagen's DNAeasy Blood and Tissue Kit (Gen students) and a modified Chelex procedure (GenEvo students). The Chelex procedure provides a cheap alternative to commercially produced kits. Students enrolled in the Gen course amplified 10 microsatellite loci via polymerase chain reaction (Butler et al., 2014). Microsatellites are repetitive regions of noncoding DNA that can be used to study population genetics. Students enrolled in the GenEvo course amplified a fragment of the cytochrome oxidase I (COI) gene (see Table 2 for an alternative schedule for COI analysis; Folmer et al., 1994). For the microsatellite loci, we found it easier to assign individuals (or pairs) 8–16 DNA samples to amplify at one or more loci. We preloaded DNA into strip tubes and had students prepare and aliquot the master mix. For the COI fragment, each student prepared a reaction to amplify their extracted DNA. Amplified DNA was genotyped (microsatellites) or sequenced (COI fragment) by Eton Bioscience (www.etonbio.com). We completed the cycle sequencing reaction before submitting samples to Eton; however, Eton can complete this task as well for an additional charge. Costs of genotyping and sequencing can be estimated by creating an account with Eton. Microsatellite loci were separated on an Applied Biosystems 3730xl DNA Analyzer and sized with their 500 LIZ size standard by Eton. Genotypes were assigned using the default parameters on PeakScanner 2.0 (Thermo Fisher Scientific). We provided our students with cleaned and trimmed COI sequences. Microsatellite DNA and COI sequences were analyzed by students in groups of two. Data was shared across the entire class for more robust analysis. Students also used barcode results to verify the accuracy of our dichotomous key. Detailed procedures for the genetics portion of the class can be found in Appendices 6–12).

Assessments

We used the following assessments to gain a better overall understanding of how our students' attitudes toward science, perceptions of environmentalism, research skills, and soft skills (communication and presentation) changed after participating in the CURE. Students completed five types of assessments over the course of the semester: scientific attitude inventory, new ecological paradigm scale, skills assessment, poster project, and peer evaluations. If you have time, you can include all the assessments, or you can choose the ones that best fit your learning objectives.

Scientific Attitude Inventory II (SAI II). The SAI II is a questionnaire designed to understand a person's ideas about science (Moore & Foy, 1997). Students completed this assessment along with the NEP-R (described ahead) at the beginning and end of each semester. The assessment was used to identify changes in students' overall scientific attitude. The SAI II consists of 40 items across four categories: interests in science, attitudes toward science, views of scientists, and desires to become scientists.

New Ecological Paradigm Scale (NEP-R). The NEP-R is a revised version of the NEP (originally published in 1978), assessing people's environmental orientation (Dunlap et al., 2000). Students completed this along with the SAI II at the beginning and end of each semester. The assessment was used to identify changes in their environmental orientation. The NEP-R consists of 15 items across five categories: balance, eco-crisis, anti-exempt, limits, and anti-anthro. As part of their finals week assignment, students enrolled in EnvBio at the PUI compared their personal responses on the pre/post SAI II and NEP-R and then wrote a reflection (~1 page, double-spaced) on how their attitudes changed and what they thought caused the change.

Skills assessment. Students completed a skills assessment following each lab to reflect on the activities for the day. The assessment was used to give students an understanding of how their skills developed over the course of the project and to allow reflection on how participation in the project impacted different science skills utilized in the project (Appendix 13). The skills assessment asked the following questions: What new skills have you learned this week? What skills have you honed/improved this week? What was the easiest part of the project this week? What was the hardest part of the project this week? What suggestions do you have for improving this week's project activities?

Poster project (act like a scientist). Students created posters in groups addressing a research question that their specific class studied (related to ant biodiversity). Posters were presented to the respective classes. Peers provided feedback that the groups used to improve their posters before final submission. (The rubric is in Appendix 14).

Peer evaluations. There were two types of peer evaluations used over the course of the semester. We had students use the poster project rubric to evaluate their peer's posters. These peer evaluation scores were averaged and used with the instructor score to provide an overall poster score for students. We also had students evaluate themselves and their group members for participation in individual parts of the project throughout the semester. Peers were asked to score group members on a scale of 0–4 (never–always) on four different factors and two open-ended response questions so we could determine the extent to which each group member contributed to the project and so they could practice identifying strengths/weaknesses in others (Appendix 15).

○ Summary

Students who completed these activities were able to explore different ways of learning about ecosystems, biodiversity, and/or barcoding. Students got practice working in large and small groups and individually to complete various aspects of a research project. They were able to learn communication skills in addition to research skills that are transferable to other classes. Anecdotally, students reported that they really liked working on a project in real time. Their results were shared with the Southern Utah Bureau of Land Management office, and their data was used in a publication for an independent research project. Overall, participation in a semester-long, multi-institution project is an interesting way to share science with students and get them interested in real-world problems.

○ Supplemental Material

The following appendices are available with the online version of this article:

Appendix 1—Ant Collection Procedure: Aspirators
Appendix 2—Ant Aspiration Data Sheet
Appendix 3—Key to the Common Ant Genera of Southern Utah
Appendix 4—North Carolina Dichotomous Key
Appendix 5—Ant Identification Data Sheet
Appendix 6—DNA Extraction
Appendix 7—PCR (Microsatellites)
Appendix 8—PCR (Cytochrome Oxidase I)
Appendix 9—Gel Electrophoresis
Appendix 10—Cycle Sequencing
Appendix 11—Analyzing Microsatellite Data
Appendix 12—Analyzing COI Data
Appendix 13—Skills Assessment
Appendix 14—Poster Rubric
Appendix 15—Peer Evaluations

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CARRIE JO BUCKLIN (carriebucklin@suu.edu) is an associate professor at Southern Utah University, Cedar City. LAURIE MAUGER (lmauger@21pstem.org) is a university-level STEM education assessment and curriculum specialist at 21st Century Partnership for STEM Education, Conshohocken, PA.