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Taking Temperature with Leaves: A Semester-Long Structured-Inquiry Research Investigation for Undergraduate Plant Biology

PAIGE E. COPENHAVER-PARRY

ABSTRACT

Inquiry- and course-based research pedagogies have demonstrated effectiveness for preparing undergraduate biology students with authentic scientific skills and competencies, yet many students lack the experience to engage successfully in open-ended research activities without sufficient scaffolding and structure. Further, curricula for student-centered laboratory activities are lacking for several biological disciplines, including plant biology and botany. In this article, I describe a semester-long structured-inquiry research curriculum developed for a plant biology course taught to second-year biology students that integrates key elements of inquiry and discovery while providing a structured approach to gaining research skills. In the research project, students collect leaves from woody dicot plants across a range of environments that are characterized by different mean annual temperatures, and investigate the relationship between various leaf characteristics and temperature. Curricular materials are provided to teach skills in scientific paper reading, field data collection, data processing including microscopy and

image analysis, quantitative data analysis in R, biological inference, and scientific writing. This comprehensive, ready-to-implement curriculum is suitable for plant biology, botany, and plant ecology courses and is particularly valuable for students with no prior research experience.

Key Words: botany; climate; course-based undergraduate research experience; curriculum; dicots; ecology; ecophysiology; plant biology; regression.

\circ Introduction

Recent efforts to transform and advance undergraduate biology education have consistently highlighted the benefits of transitioning laboratory exercises from traditional "cookbook" approaches to inquiry- and

research-based pedagogies (NRC, 2003; AAAS, 2011). Such efforts are intended to teach students how to practice science authentically by engaging them in opportunities to utilize the thinking strategies

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of scientists, and emphasize the open-ended, discovery-oriented nature of investigations leading to unknown results (Chinn & Malhotra, 2002; Wallace et al., 2003; Weaver et al., 2008; Gormally et al., 2009; Kloser et al., 2011). Inquiry-based laboratories and course-based research experiences have been directly linked to positive outcomes, including gains in research skills, science literacy, ability to transfer knowledge, persistence in science, improved selfefficacy, and greater inclusion of underrepresented groups (Norris et al., 2003; Gormally et al., 2009; Bangera & Brownell, 2014; Corwin et al., 2015). Despite these clear benefits, there are many barriers to implementing inquiry-based and research pedagogies in undergraduate biology courses, particularly the challenges inherent in transitioning students to open-ended activities (Weaver et al., 2008). This transition is often fraught with frustration as students encounter

> the challenges of addressing open-ended questions without a step-by-step blueprint for progress and success (Gormally et al., 2009). Without a scaffolded approach to transitioning between these two diverse laboratory approaches, students may simply lack the knowledge, experience, and abstract thinking capacities to fully engage in authentic science practices (Lawson, 1980; Purser & Renner, 1983; Weaver et al., 2008; Gormally et al., 2009). Kloser et al. (2011) outline several guidelines for effective, course-based research activities that may provide sufficient structure to support this transition. They include (1) limited technical expertise required to collect data; (2) regular checkpoints to provide feedback and correct mistakes; (3) projects that include many possible variables to enable students to shape their investigations within a constrained scope; and (4) assessments that mimic authentic research dissemination, such as

a final paper formatted according to the standards of a scientific journal. These recommendations, combined with a more structured inquiry approach in which key dimensions of the investigation are determined

by the instructor, may strike a balance between student exploration and supportive instruction, and may potentially ease the transition from traditional laboratories to authentic research (Weaver et al., 2008; Auchincloss et al., 2014).

Here, I present a research curriculum developed for a second-year plant biology course that integrates key elements of inquiry and discovery while providing a structured approach to gaining research skills and scientific competencies. This curriculum was designed to transition students from the "cookbook" labs they encounter in their first-year general biology sequence to independent course-based research projects in their third and fourth years, and to address the perceived lack of well-developed, inquiry- and research-based curricula for plant biology and botany courses. The curriculum is implemented as a semester-long research project that incorporates many pedagogical approaches with demonstrated effectiveness for increasing student engagement, retention, and application of scientific information, including outdoor education and fieldwork (Easton & Gilburn, 2012), experiential learning (Bauerle & Park, 2012), and project-based ecology (McCann & Miller, 2004; Purcell et al., 2007; Hall & Bauer-Armstrong, 2010), as well as key elements of authentic research and inquiry-based learning. The curriculum also incorporates a strong quantitative reasoning component that involves statistical modeling and R programming, which met a course objective of

improving quantitative knowledge, data analysis skills, and confidence in applying quantitative methods to biological data.

This curriculum was implemented in the laboratory section of a second-year plant biology course at George Fox University, a private, primarily undergraduate liberal arts institution with a current undergraduate enrollment of 2389. The course consisted of one section of 17 biology majors, all of whom have completed a two-semester general biology sequence. Prior education on plant biology themes was minimal for all students. The laboratory curriculum was implemented in a three-hour weekly lab period over 14 weeks, with two Saturday field trips replacing two of the weekly lab meetings. The central aim of the laboratory curriculum is to investigate what leaf morphological characteristics are most strongly associated with mean annual temperature. The curriculum addresses nine learning outcomes directly tied to Vision and Change concepts and competencies (AAAS, 2011; Table 1).

O Background

Variation in morphological characteristics arises from three primary processes: natural selection, phenotypic plasticity, and developmental programs and pathways. In each of these cases, environmental

Table 1. The curriculum is designed around nine specific learning outcomes that correspond with four (of five) core concepts for biological literacy and five (of six) core competencies for disciplinary practice as outlined by the Vision and Change framework for undergraduate biology education (AAAS, 2011).

Learning Outcome	Concept	Competency
Follow the process of science to develop an evidence-based hypothesis grounded solidly in scientific literature, collect data according to a sampling design that ensures sufficient representation, and generate meaningful conclusions from data that directly address the hypothesis and research question and reflect a mechanistic understanding of the observed trends in data.		Process of Science
Use a species identification guide and other resources to accurately identify deciduous tree and shrub species.	Evolution	Process of Science
Prepare plant herbarium specimens that are carefully and correctly preserved.		Process of Science
Effectively and accurately use computational tools including ImageJ and R to collect and analyze data.		Interdisciplinary Nature of Science
Access and utilize publicly available climate data from an online database.		Quantitative Reasoning; Modeling and Simulation
Design and conduct a proper regression analysis that directly addresses the research question, tests the hypothesis, and is correctly interpreted and presented in the context of the research question and hypothesis.		Quantitative Reasoning; Modeling and Simulation
Clearly articulate how morphology-climate relationships reflect underlying environmental constraints on physiology.	Structure and Function; Energy and Matter; Systems	
Understand and articulate how variations in plant characteristics reflect evolutionary responses to the environment	Evolution; Energy and Matter; Systems	
Correctly communicate the motivation, process, results, and implications of the research in a format and style that are consistent with scientific standards.		Communicate and Collaborate

conditions exert some limit on physiological processes, giving rise to specific morphological characteristics (Niklas, 1992; Little et al., 2010). Plants have an optimal range of temperatures in which they can survive. At low temperatures, plant cell contents freeze and metabolic activity is halted, and at high temperatures, proteins associated with photosynthesis and other physiological processes can be damaged or denatured and cell membrane integrity may be compromised. Leaves are the site of most temperature regulation. Leaves capture heat via solar radiation and ambient heat and lose heat primarily via evaporative water loss through transpiration. In order to maintain ideal temperatures in the diverse habitats that plants occupy, plants have evolved a variety of leaf traits that influence their ability to capture and lose heat, including variation in leaf surface area, leaf margin characteristics, stomatal density, and leaf shape (Leuzinger & Korner, 2007). For example, we might expect that a plant in a hot environment would benefit from high stomatal density, which allows for greater evaporative water loss, and low surface area, which reduces heat capture via radiation. However, if the hot environment is also dry, a plant with high stomatal density might be at risk of dehydration. In such an environment, heat loss may occur primarily by sensible heat transfer, with net convective heat loss increasing with leaf surface area. This example underscores the trade-offs between resource availability and temperature maintenance that are reflected in leaf morphology (Givnish, 1984; Bloom et al., 1985). In reality, leaves must balance multiple physiological requirements simultaneously, often resulting in complex combinations of leaf characters that vary strongly with climate (Yang et al., 2015). Climate-induced variation in leaf morphology is particularly pronounced and detectable among woody dicotyledonous plants (trees and shrubs; hereafter referred to as "woody dicots").

In this research project, students will collect leaves from woody dicot plants across a range of environments that are characterized by different mean annual temperatures, and investigate the relationship between various leaf characteristics and temperature to answer the following research question: What leaf morphological characteristics are most strongly associated with mean annual temperature?

Methods

Delivery of the curriculum proceeds through 14 weeks of lab meetings that include opportunities for instruction, field data collection, sample processing, data analysis, and paper writing (Table 2). All curricular materials, including protocols, rubrics, and worksheets, are freely available online [\(https://figshare.com/projects/Curriculum_for_Taking_](https://figshare.com/projects/Curriculum_for_Taking_temperature_with_leaves_A_semester-long_structured_inquiry_research_investigation_for_undergraduate_Plant_Biology/62609) [temperature_with_leaves_A_semester-long_structured_inquiry_](https://figshare.com/projects/Curriculum_for_Taking_temperature_with_leaves_A_semester-long_structured_inquiry_research_investigation_for_undergraduate_Plant_Biology/62609) [research_investigation_for_undergraduate_Plant_Biology/62609\)](https://figshare.com/projects/Curriculum_for_Taking_temperature_with_leaves_A_semester-long_structured_inquiry_research_investigation_for_undergraduate_Plant_Biology/62609).

The curriculum is designed to be completed by teams consisting of four or five students. Formative assessments including the pre-lab, paper-reading guide, data analysis homework, and biological inference table are completed individually, while summative assessments – the research paper and herbarium – are completed by research teams.

Week 1: Project Overview & Scientific Paper Reading

In the first week, students are introduced to the research project and learning outcomes. Students must complete a pre-lab assignment that includes reading the project overview and predicting how five leaf morphological traits might vary between warm and cool environments (Table 3). During the lab period, students will develop additional background knowledge by reading a recent paper describing a global evaluation of leaf morphology–climate relationships (Yang et al., 2015). Because many of the students engaging in this curriculum are relative novices at reading scientific articles, they may lack strategies for effective and critical reading and may also struggle to identify relevant scientific articles (Greenhalgh, 2001). This exercise addresses these challenges by providing an initial paper and structuring the reading with a reading guide. To implement the reading guide, student volunteers each read a paragraph of the paper aloud to the class, starting with the introduction. At the end of each section, students are encouraged to reread the text silently and then complete the relevant portion of the reading guide. Students then discuss their responses with a peer and revise as necessary. When all pairs have compared and revised responses, the section is debriefed as a class. This process is continued through the end of the paper, ending with the abstract, which allows students to use the abstract to test their understanding of the rest of the paper. This section-by-section approach interrupted by sharing with peers has been indicated to increase student confidence and understanding of material when applied in other undergraduate biology contexts (Bogucka & Wood, 2009).

Weeks 2, 3, 5, 6, 7, 8: Field Data Collection & Sample Preparation

In these weeks, students participate in field trips to collect leaf samples. Field trips should include visits to sites that span a gradient of mean annual temperatures; for example, the six trips organized at George Fox University involved data collection at sites extending from the west slope of the Cascade mountain range to the Oregon coast. Based on analysis of leaf morphology at these sites, a mean annual temperature gradient of 2.0–2.5°C appears sufficient to identify significant leaf morphology–climate relationships. In regions with limited topographic relief, sufficient temperature gradients may be found in association with large drainage basins, such as in the Great Lakes region of the United States (Lofgren, 2004). Data collection in week 2 was conducted on campus to allow lab time to review protocols for field data collection, stomata casting, leaf surface area analysis, leaf character scoring, and leaf specimen preservation. Visits to the most distant locations were accomplished by scheduling two full-day field trips on Saturdays during the semester; these trips replaced the regular lab meeting, and three sites were visited on each of these trips. Students were also required to collect at one additional site with their lab group, outside of the lab period, for a total of 11 sampling sites that varied in mean annual temperature. Note that collecting permits may be required for some locations.

During field-trip weeks, students are expected to process and prepare leaf samples outside of lab hours. Processing is initiated by using the CLAMP (Climate Leaf Analysis Multivariate Program) scoring protocol to score leaves according to 10 leaf characteristics (Table 4; Wolfe, 1990). CLAMP is a widely utilized correspondence analysis program for deducing relationships between climate and leaf physiognomy in woody dicots that includes scoring protocols for quantifying leaf physiognomic variables (Wolfe, 1990). Although this project does Table 2. A suggested pacing guide for the semester-long curriculum organized into 14 weeks of instruction in three-hour laboratory periods. The curricular materials associated with each activity are shown. All curricular materials are available at https://figshare.com/projects/Curriculum_for_Taking_temperature_ [with_leaves_A_semester-long_structured_inquiry_research_investigation_for_undergraduate_Plant_](https://figshare.com/projects/Curriculum_for_Taking_temperature_with_leaves_A_semester-long_structured_inquiry_research_investigation_for_undergraduate_Plant_Biology/62609) [Biology/62609](https://figshare.com/projects/Curriculum_for_Taking_temperature_with_leaves_A_semester-long_structured_inquiry_research_investigation_for_undergraduate_Plant_Biology/62609).

Table 2. Continued

Table 3. As a part of the pre-lab activity for week 1, students complete a table that includes several of the leaf characteristics they will measure to include predictions for what variation of each trait might be found in a warm environment versus a cool environment. Example student responses are shown.

Table 4. Leaf samples collected at sites that vary in mean annual temperature are scored for 10 morphological characteristics according to the CLAMP protocol (Wolfe, 1990) to translate traits into quantitative data.

not utilize the correspondence analysis, it uses the scoring protocol to translate leaf characteristics to quantitative data. Processing leaf samples also requires creating stomata casts and determining stomatal density, measuring leaf surface area by analyzing digital scans with the ImageJ computer program (Rueden et al., 2017), pressing leaves, and mounting pressed leaves for proper herbarium preservation. The methods are demonstrated for students in week 2; all protocols are available with the full curriculum.

Week 4: Introduction to Scientific Writing

In the fourth week, students are provided with lab time to begin constructing hypotheses, reviewing literature, and writing the introduction sections of their research papers. This is an ideal opportunity to provide early feedback on research papers. Kloser et al. (2011) suggest that effective integration of research experiences in undergraduate courses benefits from assessments that correspond with the dissemination methods used by practicing scientists, such as final research papers that follow the format of a relevant scientific journal, and highlight the importance of providing multiple iterations of feedback. For these reasons, this curriculum introduces the paper-writing process early in the semester. Because most students will likely be novices at scientific writing, substantial instructional guidance may be needed. This curriculum provides some of this guidance through a detailed guidelines document and grading rubric that follow the requirements of the journal Ecology (available with full curriculum). Many additional resources are available for teaching science writing and have been used successfully with this curriculum (e.g., McMillan, 2017).

Week 9: Accessing Publicly Available Climate Data

In the ninth week, students utilize a publicly available climate database and extract mean annual temperature (MAT) estimates that correspond with their collection locations. These data will later be used to evaluate relationships between MAT and leaf characteristics. Data are accessed from ClimateWNA, a downscaled climate data set for North America based on PRISM data (Daly et al., 2008) that is available through a user-friendly web interface (Wang et al., 2012). Engaging students in data extraction achieves a variety of learning outcomes related to handling of big data. As questions in biology become more data-rich than ever before, training in data science has been increasingly emphasized as a necessary skill for all students (NRC, 2009; AAAS, 2011; Hampton et al., 2013; NAS, 2018). While ClimateWNA data are easy to access and straightforward to organize and manipulate, this activity provides an opportunity for students to learn about large climate data sets and their many uses and purposes, and to interact with one such data set. Instructions for accessing ClimateWNA data are included with the full curriculum.

Weeks 10 & 11: Data Analysis

In these weeks, students engage in interactive R programming courses delivered through the swirl package (Kross et al., 2017) to learn basic R programming, data handling, and simple linear regression. This component of the curriculum is designed for students with no prior exposure to R programming or statistical analysis, and meets a major educational objective of enhancing programming, quantitative reasoning, and data analysis skills. Training in data analysis and programming is among the most pressing unmet needs in science, and has been repeatedly emphasized in leading models for undergraduate biology education (NRC, 2003; AAAS, 2011; Barone et al., 2017; NAS, 2018). Challenges ranging from limited instructor expertise to student math anxiety have slowed progress on addressing this need

(Donovan, 2008; Uttl et al., 2013). The swirl package was designed to mitigate many of these challenges and to present students with authentic programming and analysis tasks in a research-based context, using a language that is used extensively in scientific research (Brown & Wilson, 2018). An interactive platform for learning and teaching R in the RStudio console, the swirl package simplifies the R learning process by providing a guided, interactive experience through on-screen prompts and exercises that students answer directly in R. It seamlessly integrates learning of biology content, programming, and data analysis, and contributed lessons and consoleguided implementation mitigate the need for instructors, who may lack confidence in teaching statistics or programming, to create or assess original coding and statistics materials. Anecdotally, the use of custom swirl courses in all of my biology courses has dramatically enhanced the speed and success with which students learn to implement statistical analyses in R.

This curriculum includes two swirl lessons, instructional handouts, and assignments that guide students through basic R programming, data handling and visualization, and linear regression. The linear regression assignment will require students to construct a series of simple linear regressions to quantify relationships between mean annual temperature and leaf characteristics, and to extract coefficients and measures of uncertainty. At the end of the exercises, students will generate a table with the results of their regressions (Table 5), which will comprise the analysis for their research papers and form the foundation for all subsequent inference. The lessons are implemented using RStudio Cloud ([https://rstudio.cloud/\)](https://rstudio.cloud/).

Weeks 12 & 13: Biological Inference

In these weeks, students translate the results of their linear regression models to biological inferences and explore mechanisms to explain their findings. The analytical and mechanistic thinking required to translate numerical output to biological conclusions supported by rational, evidence-based explanations is typically not considered intuitive, but is a critical step in transitioning from passive learning to true scientific thinking (Kitchen et al., 2003). Evidence-based, mechanistic explanations for analytical results will also comprise much of the discussion section of the final papers. For this component of the research project, guidance for generating inference is provided through an inference table (Table 6), which requires students to provide mechanistic explanations for major quantitative findings from their regression analyses supported by citations from primary scientific literature. I have also found it helpful to require checkpoint meetings with individual research teams during these weeks to discuss their inference tables and provide additional feedback as students construct their research papers.

Week 14: Final Projects

In the final week of the project, research teams submit a complete scientific research paper that follows the guidelines outlined in the writing guide and paper rubric available with the full curriculum. Research teams also submit a complete herbarium consisting of at least 150 correctly preserved woody dicot leaf samples collected throughout the course of the project. In lab, research teams informally present their findings, and the instructor guides Table 5. Students analyze data by constructing simple linear regressions of site-level means of each leaf characteristic against mean annual temperature. Students report relevant statistics resulting from these regression models in this regression table, which includes all quantitative output necessary to report in research papers. Example output from regressions on student data is shown here.

Response Variable	Slope	SE	95% Confidence Interval	Effect
Stomata density	21.00	7.51	6.29 to 35.71	$+$
Surface area	-14.36	6.07	-26.25 to -2.47	
Lobing	0.01	0.07	-0.10 to 0.16	$\overline{0}$
Teeth	-0.08	0.03	-0.13 to -0.02	
Tooth spacing	0.02	0.03	-0.05 to 0.08	$\overline{0}$
Tooth closeness	0.02	0.03	-0.04 to 0.07	Ω
Teeth round	0.06	0.03	0.01 to 0.12	$+$
Teeth acute	0.03	0.04	-0.04 to 0.10	0
Teeth compound	0.09	0.04	0.01 to 0.17	$+$
Apex form	-0.01	0.01	-0.03 to 0.02	Ω
Length:width	-0.01	0.04	-0.09 to 0.08	Ω
Shape	0.07	0.04	-0.01 to 0.15	0

Table 6. To facilitate translation of numerical output to biologically meaningful conclusions, students complete this inference table in week 12, which acts as a tool to guide the development of mechanistic explanations for specific findings. Example student responses are included here.

a reflective discussion of the class's collective findings. Reflecting on research through writing and discussion has been demonstrated to increase students' critical thinking skills in inquirybased laboratory contexts (Gupta et al., 2015). Reflections may focus on inconsistencies in findings across groups and potential explanations, study design, errors, applications, and implications of the findings, as well as skills and knowledge gained through participation in the research project.

\circ Conclusions

This curriculum describes a scaffolded approach to transitioning between traditional laboratories and inquiry-based investigations while providing all the benefits of student engagement in authentic research. Structure is provided through an extensive curriculum that includes a predetermined research aim, protocols for data collection and processing, and exercises to teach key research skills. Inquiry is supported through the many leaf character–climate relationships available to be explored, which provide flexibility for students to generate unique hypotheses within helpful constraints, and through engagement in analysis and inference on data sets with unknown outcomes and results. Additionally, the research project allows students to gain skills and content knowledge related to plant biology. All students who completed this curriculum found it to be valuable – and, although this is anecdotal, they have also been perceived to exhibit greater preparedness for research in upper-division courses. The curriculum is particularly valuable for students with exclusive prior exposure to cookbook labs and offers a ready-to-implement curriculum for courses in plant biology, botany, and plant ecology. Future implementations of this curriculum will include an assessment of the degree to which students' conceptual understanding of plant biology is improved and their appreciation of plants is enhanced through participation in the research project.

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