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CourseSource

Lesson

Using Images of Foraging Leaf-Cutter Ants to Teach Linear Regression

Ariel Firebaugh[1], Justin Touchon[2], Hayley Orndorf[3], and Jeremy Wojdak[1*]

[1]Radford University

[2]Vassar College

[3]University of Pittsburgh

Abstract

This lesson offers students a realistic, open-inquiry research experience, even when lab or field research is not possible. We first introduce students to leaf-cutter ants and how they forage. Then we provide images and videos of foraging leaf-cutter ants from a rainforest in Panama. Students develop a hypothesis, propose a study, and collect primary data using image analysis software. Because students have ownership of their research study, they become invested in the questions they posed. Curiosity motivates students to learn linear regression and apply it to their data. Students report positive experiences with the lesson and a greater interest in learning statistics in this context. Faculty report that students enjoy the lesson and that it runs smoothly. We conclude that this lesson provides an enjoyable and effective approach to teach a fundamental analytical skill.

Citation: Firebaugh A, Touchon J, Orndorf H, Wojdak J. 2020. Using images of foraging leaf-cutter ants to teach linear regression. *CourseSource*. https://doi.org/10.24918/cs.2020.32

Editor: Nathan Emery, Michigan State University

Received: 9/18/2019; **Accepted:** 6/17/2020; **Published:** 9/10/2020

Conflict of Interest and Funding Statement: This lesson and manuscript were funded by a grant from the National Science Foundation (DUE-IUSE 1431671 AIMS: Analyzing Images to learn Mathematics and Statistics). None of the authors has a financial, personal, or professional conflict of interest related to this work.

Supporting Materials: Supporting Files S1. Leaf-cutter ants – Alternative teaching strategies; S2. Leaf-cutter ants – Student handout; S3. Leaf-cutter ants – Introduction video; S4. Leaf-cutter ants – External resources; S5. Leaf-cutter ants – Conducting linear regression in Excel; S6. Leaf-cutter ants – Conducting linear regression in JMP; S7. Leaf-cutter ants – Conducting linear regression in R; S8. Leaf-cutter ants – ImageJ and regression videos; S9. Leaf-cutter ants – Regression practice sheet; S10. Leaf-cutter ants – Regression assessment; S11. Leaf-cutter ants – Instructor slides; S12. Leaf-cutter ants – Presentation rubric; and S13. Leaf-cutter ants – Alignment with AP Biology, Common Core, and NGSS standards.

*Correspondence to: Box 6931, Radford University, Radford, VA 24142-6939. Email: jmwojdak@radford.edu.

Learning Goal(s)

From the Ecology Learning Framework:

- How do organisms mediate the movement of matter and energy through ecosystems?
- How do organisms obtain and use matter and energy to live and grow?
- How do species interact with their habitat?

From the Science Process Skills Framework:

- Reading research papers
- Reviewing prior research
- Asking a question
- Formulating hypotheses
- Gathering data/making observations
- Analyzing data
- Displaying/modeling results/data
- Interpreting results/data
- Communicating results

Learning Objective(s)

Students will:

- form hypotheses based on prior information and their own observations.
- design a scientific study to address a focal hypothesis.
- use image analysis software to generate data from an image set.
- conduct and interpret linear regression analyses.
- present their proposed study and results to peers.

INTRODUCTION

Motivating student learning of quantitative skills

A compelling and meaningful research context can help motivate students to learn quantitative skills (1). We believe images are one way to provide this context and to facilitate student learning. Images stir our imagination. They stimulate our powers of observation and inquiry. The power of image analysis to aid instruction already has a foundation in the pedagogical literature for mathematics, geo-engineering, and computer science (2-5), and is now becoming more common in biology (see examples of teaching activities such as 6-9, https://www.biointeractive.org/classroom-resources/lizard-evolution-virtual-lab, https://cellprofiler.org/outreach, https://www.biointeractive.org/classroom-resources/scientific-inquiry-and-data-analysis-using-wildcam-gorongosa).

This lesson uses images as a framework for teaching teach quantitative and analytical skills. After a quick introduction to the study organism and to image analysis, students explore real biological phenomena using sets of photos and videos. Students develop research questions and hypotheses, then collect and analyze data from the images to test their hypotheses.

When we have taught this lesson, we have observed that students seem to understand data they have collected themselves better than pre-collected data from textbooks or problem sets. Students know where the data come from, literally see variation among measurements, and problem-solve to reduce measurement error. By the time our students analyze their data, they already have intuitive expectations for a sample's mean and variance, the relationships among variables, and the results of hypothesis tests or regression analyses. We believe that when students compare their expectations with the actual results of the analyses, they deepen their understanding of statistics and their understanding of the study system.

Context

The lesson centers on images and videos of leaf-cutter ants, Atta columbica, from the Neotropics. Leaf-cutter ants trim leaf pieces from nearby vegetation, and bring the leaves to their nest to feed a fungus, from which the ants themselves feed. They can live in colonies of several million individuals, and divide work among an array of castes, including scouts, foragers, garbage disposers, nannies, soldiers, and queens. Leaf-cutter ants have featured in numerous nature documentaries and magazines (e.g., https://www.bbc.co.uk/programmes/p003xjc2, http://naturedocumentaries.org/2954/life-inside-colony/). As Cameron Currie, who studies the fungus-growing ants said, "*Anyone who has ever come across a trail of ants cutting leaves and watched that trail run through the forest can recognize how charismatic [they are], and what kind of large impact they have on tropical ecosystem in which they occur.*" (https://www.nsf.gov/news/mmg/mmg_disp.jsp?med_id=72352)

In our experience, leaf-cutter ants' foraging habits, colonial social structure, and fungal farming intrigue students. Students quickly begin to wonder about how ants decide what plants to cut, why some ants cut larger leaf pieces and other ants cut smaller leaf pieces, how ants partition work, and how ants navigate the forest floor. The research on leaf-cutter ant foraging contains some interesting twists and turns (e.g., 10-13). The "obvious" answers aren't often correct. For example, since the ant colony is usually considered the unit of natural selection, selection should favor maximization of the colony-level leaf foraging rate rather than an individual ant's. How this plays out in terms of ant behavior, leaf fragment size, and traffic patterns along foraging trails is far from simple.

The primary learning objectives in this lesson are quantitative skills like linear regression and fundamental scientific practices as called for in Vision and Change (14). Students will form hypotheses, design a scientific study to address a focal hypothesis, use image analysis software to generate data from an image set, conduct and interpret linear regression analyses, and present their proposed study and results to peers. In sum, this lesson allows students to complete core scientific practices while conducting research on a tropical insect (in their own classrooms!).

Intended Audience

We designed this activity to fit into an introductory biology course's units on ecology, evolution, or animal behavior, or advanced courses in any of those fields. Authors JW and AF have collectively taught this module in many lab sections of an introductory ecology course with ~24 students per section. This course is typically taken by first-year students. We teach this module over one or two ~three-hour lab periods. We suspect an expanded version of the lesson could lead to full research papers or posters for more rigorous research projects. The activity could also provide a context for analysis in a biostatistics course.

Required Learning Time

The lesson is easy to expand, condense, or modify as desired. It is suitable for lecture, lab, or as homework - as long as computers are available to students. The authors and colleagues have taught this module several different ways in a number of different contexts. Most recently, AF taught this lesson over two consecutive ~three-hour lab periods. This approach allowed students more time to develop hypotheses and present their study plans and final results. We describe AF's implementation here, but suggest alternative implementations in the Teaching Discussion and in Supporting File S1. Leaf-cutter ants – Alternative teaching strategies.

Prerequisite Student Knowledge

Reading the student handout (Supporting File S2. Leaf-cutter ants – Student handout) and watching the associated video (Supporing File S3. Leaf-cutter ants – Introduction video) will give students enough background information to develop reasonable hypotheses, collect data, and perform analyses. When time permits, we find assigning primary literature articles about leaf-cutter ants helps students develop more biologically meaningful hypotheses. We provide four primary research articles about leaf-cutter ants at the end of the student handout and in Supporting File S4. Leaf-cutter ants External resources. We chose these articles because they are relevant and digestible for beginning science students. Having students read even excerpts can be helpful.

Students will need to use at least two programs to extract, visualize, and analyze data: the freely available image analysis software ImageJ and the statistics software of the instructor's choosing. We assume students will have little or no experience with ImageJ, and so provide step-by-step instructions to help navigate the software (page 3 of Supporting File S2. Leaf-cutter ants – Student handout). There is a learning curve, but most students become proficient after a few minutes of practice. We also include instructions for creating scatterplots and conducting regression analyses in Excel (Supporting File S5. Leaf-cutter ants – Conducting linear regression in Excel), JMP (Supporting File S6. Leaf-cutter ants – Conducting linear regression in JMP), and R (Supporting File S7. Leaf-cutter ants – Conducting linear regression in R). Prior experience making figures will help students complete the lab more quickly, but is not mandatory. Students in our introductory biology courses vary a great deal in proficiency with computers and with graphical visualization of data. Some students can create publication-quality figures with ease; other students need practice using copy and paste functions in Excel. Grouping students with higher/lower proficiency encourages them to help each other instead of waiting for instructor guidance. As a result, all groups move through the lab more quickly. However, it may be necessary to provide prompts or structures encouraging equal division of work among group members. For example, AF states that she expects all group members to help create scatterplots, and requires students to turn in a log of specific tasks they completed (e.g., proofreading scatterplot, reviewing online tutorials to solve technical issues).

Video tutorials for ImageJ and Excel empower students to become their own tech support, and free the instructor from repeating the same instructions to multiple groups. Video tutorials on performing basic image analysis tasks and conducting/interpreting regression analyses can be found in Supporting File S8. Leaf-cutter ants – ImageJ and regression videos.

Students will need a working knowledge of basic statistics to interpret their results. They will need to know how to conduct linear regression and interpret the regression equation, *p*-value, and R^2 value. Since most students in our introductory biology classes have never taken a statistics course, we developed the aforementioned video tutorials (Supporting File S8. Leaf-cutter ants – ImageJ and regression) and a two-page activity (Supporting File S9. Leaf-cutter ants – Regression practice sheet) to help them interpret scatterplots and regression results. The regression practice and instructional videos prime the students to have clearer expectations for their own analyses. Students should complete the practice exercise before analyzing data for this module.

Prerequisite Teacher Knowledge

Previous experience with image analysis is not required. Basic understanding of linear regression, hypothesis testing, and data visualization is required, though instructors can find helpful reminders in the student materials. Reading a few primary articles about leaf-cutter ants can help broaden the instructor's perspective of the research context and the interesting questions students might pose. We suggest a few primary literature articles in Supporting File S4. Leaf-cutter ants – External resources. Additionally, instructors may wish to review the basics of linear regression. We include a short list of resources in Supporting File S4. Leaf-cutter ants – External resources.

SCIENTIFIC TEACHING THEMES

Active Learning

Students actively engage with real scientific practices throughout the lesson as they imagine and then conduct a modest but complete study. Students develop research questions, refine those questions into testable hypotheses, propose a study, collect real data, statistically analyze the data, interpret analyses, and present their results and conclusions. Depending on how the lesson is implemented, there can be virtually no lecturing or didactic instruction. Throughout day one, students are prompted to engage with the material by writing down prior knowledge, brainstorming research questions, and making predictions.

Assessment

We assess students' ability to write testable scientific hypothesis and design methods when small groups propose their studies to the class during the first lab period. Peer and instructor feedback helps students refine their ideas during this formative process. During the second lab period, students summarize their data, conduct analyses, and present their results. A worksheet reinforces the general principles of regression analysis (Supporting File S9. Leaf-cutter ants – Regression practice sheet). Student knowledge of linear regression can also be measured with a short assessment (example provided in Supporting File S10. Leaf-cutter ants – Regression assessment).

Inclusive Teaching

An accessible "indoor" field experience

This computer-based lesson gives classes/courses who are unable to go into the field because of geographic, budgetary, medical/health, time, or seasonal/weather constraints the opportunity to collect meaningful ecological data.

Emphasis on visuals

Images and videos are powerful tools for engaging student interest. However, the visual nature of the lesson does place unfortunate limitations on the value for students with visual impairments. Many image analysis tasks done manually require careful mouse-work to delineate shapes or lengths on a screen. Thus, as written, the lesson is not well-suited for students with visual impairments.

There are modifications that could improve accessibility and benefit all students by providing other ways of encoding and interpreting visual information. By its nature, image analysis turns visual information into quantitative information - numerical measurements of lengths, areas, color intensities, etc. Providing numerical metrics of visual properties of biological specimens could help students with visual impairments experience specimens (via screen readers, sonification systems, etc.) that would otherwise be unavailable, and may benefit all students by providing multiple ways of representing or encoding the information.

Interestingly, image analysis is often automated to improve speed, accuracy, and repeatability (15). A lesson that focused on image analysis automation could teach valuable real-world skills and open up participation to more students (e.g., students in computer science courses).

Group work

Group work, when managed well by the instructor, can provide an environment where the diverse assets of students yield a better product than any individual student could have created themselves. Facilitating productive collaboration can be challenging, though, and requires active strategies of the instructor. Below are some approaches we have used successfully. We encourage instructors to choose strategies they believe will be effective to ensure equal and active participation among their students (see 16-17 for more ideas).

Gather ideas from all group members

When groups need to pick a research question, some students will be more willing to share initial ideas than others. Give students time to think about and make a list of possible research questions before they form groups. Once they are in groups, ask students to exchange lists and read each other's ideas aloud.

Have students work individually first

Although students work on most of this lab in groups, they complete the regression assignment (Supporting File S9. Leaf-cutter ants – Regression practice sheet) individually. Providing time for students to think through and write out their answers before discussing with group members helps students clarify what they understand and what they have questions about.

Diversify group skillsets

We find that when groups are heterogeneous with regards to Excel proficiency, they tend to troubleshoot technical issues internally instead of waiting for help from the instructor. This is desirable, as it encourages students to help each other and builds community.

Make group members accountable to each other (and to you!)

Two goals of this activity are to help all students become more comfortable creating scatterplots and performing linear regressions. These goals cannot be accomplished if one group member makes the scatterplot or runs the analysis while the other(s) sit passively. Tell students you expect them to actively contribute to every component of the module. For example, if one student is creating a scatterplot, their group members should be reviewing online tutorials or proofreading the graph. One strategy to keep group members accountable is to require students to write and turn in a log of the specific tasks they complete. Group members should review and sign one another's logs before submission. Another strategy is to require students to complete anonymous peer evaluations at the end of each lab.

LESSON PLAN

The authors and colleagues have taught this lesson several ways in a number of different contexts. Most recently, AF facilitated this lesson over two consecutive three-hour lab periods, and it is this implementation that is described below (for a description of a second approach we have implemented, see Supporting File 1. Leaf-cutter ants – Alternative teaching strategies). We encourage instructors to modify this lesson as desired.

Before Class

Accessing ImageJ

Decide how your students will access the ImageJ image analysis software needed to complete the activity. There are several options, all of which are free and relatively easy. ImageJ is available for download (https://imagej.nih.gov/ij/) and installation on student computers or in campus computer labs. Alternatively, ImageJ can be run in the cloud on QUBESHub, an online platform for quantitative biology education (https://qubeshub.org/tools/ImageJ). Each student will need to create a free account to run ImageJ on QUBESHub. Because ImageJ on QUBESHub is running in a virtual machine within a browser, be aware that students may have some initial confusion as to where they are in the computing environment. Whatever ImageJ access option you choose, we advise you to practice the lesson's tasks before class to help you become comfortable with the software. The biggest difference we noticed when testing the interface was in importing image files. To work with an image, it must first be uploaded via the "Import/Export" window, then opened using File Open.

Student assignments

Ask students to read the student handout (Supporting File S2. Leaf-cutter ants – Student handout) and to watch the video of ants moving and foraging in the rainforest (Supporting File S3. Leaf-cutter ants – Introduction video). The handout and video include material on the ants' social structure, foraging activity, and their relationship with a fungus species they farm.

If time permits, assign students to read part or all of a scientific article on leaf-cutter ant foraging. AF did not assign papers due to scheduling constraints, but wishes she had in retrospect. Strategies for assigning papers are described in Supporting File 1. Leaf-cutter ants – Alternative teaching strategies.

During Class

Background information: Leaf-cutter ants

Give students two minutes to write down what they already know and what they would like to know about leaf-cutter ants. Go around the room and ask each student to share one thing on their list (know/want to know). Make a big list of student responses on a white board at the front of the room.

Ask students to re-read the first section of the student handout (under the "Leaf-cutter ant" subheading), then play the introductory video (Supporting File S2. Leaf-cutter ants – Student handout) at the front of the classroom. This video ends with several intriguing "unknowns" about leaf-cutter ant biology. Tell students they will be conducting research to investigate some of these mysteries over the next two lab periods.

After students read the introduction of the handout and watch the introductory video, invite students to explore the image/video sets (available here: https://knb.ecoinformatics.org/view/doi:10.5063/F10R9MRR). Students download and watch two videos and view one image to introduce them to the ants' foraging behavior and the type of visual information available to them.

Encourage students to consider what could be learned about leaf-cutter ant foraging from a larger collection of similar images and videos. The handout guides students to think about possible data they could collect, such as measurements in one or two dimensions of ant morphology (e.g., body length, leg length, head area), the leaves the ants carry (e.g., leaf area), or the rate of movement or aspects of ant traffic. Invite students to think about possible scientific questions they could address with the data. Ask students to write down ~five questions that occur to them as they preview the videos/images.

Background information: ImageJ

Introduce students to the tool they will use to collect data: the image analysis software ImageJ. Explain that this powerful, free software was developed by the National Institutes of Health (NIH), and show examples of cell and tissue culture being measured in ImageJ (such as the example images available here: https://imagej.nih.gov/ij/docs/concepts.html). Ask students to brainstorm medical applications of image analysis (i.e., Why did the NIH spend money to develop this software?). Many introductory biology students wish to pursue careers in healthcare, so listing examples of image-based diagnostic tools (e.g., CT scans, hematographs) can help them connect the software and analysis with personally relevant biology.

Explain how ImageJ coverts pixel counts to size measurements. To do this, first show a photo of a leaf-cutter ant followed by a zoomed in version of the photo with schematized "pixels" shown (Supporting File S11. Leaf Cutter Ants – Instructor Slides). Explain that digital photos are made up of individual small pieces of information called "pixels." We can measure real distances if we know the "real world" distance a single pixel represents in the photo. That is why image analysis typically requires an object of known size (like a ruler) to be included in the image. ImageJ counts the number of pixels in a known, user-specified length, then uses that conversion factor to measure dimensions in standard units (e.g., cm, mm). For example, a user can draw a line along one centimeter along the ruler, and if that line was 100 pixels long, we (and the computer!) would know there are 100 pixels in every centimeter. Now any line, perimeter, or area can be calculated by the software by counting the pixels and using the conversion factor. Supporting File S11. Leaf Cutter Ants – Instructor Slides includes a schematic that might help students understand how ImageJ converts pixel counts to "real world" measurements.

Ask students to practice drawing lines using the Straight Line tool (see Supporting File S2. Leaf-cutter ants – Student handout for more details about how to use this tool). Once the students feel comfortable drawing lines, show them how to set a scale reference using a known length on the ruler in the photo. Ask them to practice measuring the lengths and widths of objects in the photo. Finally, show students how to use the "m" keyboard shortcut to record measurements in the results window (described on page 3 of Supporting File S2. Leaf-cutter ants – Student handout). Repeat this process to measure areas and perimeters with the Polygon tool.

Now that students know how to make and collect measurements, you may wish to lead a quick discussion about measurement error. Ask students to measure the length of the one ant carrying a leaf in the practice image. Invite students to call out their measurements or write them on the white board at the front of the room. Some measurements will be almost identical, others will differ. Prompt students to brainstorm sources of variability. Hopefully, students will recognize that measuring different sections of the ant (e.g., "antenna to tail" vs. "nose to tail") and the degree to which the image is zoomed in/out could affect their measurements. When students form research groups in a few minutes, they should discuss how to create specific, shared protocols designed to minimize measurement error.

Show students ImageJ tutorials to review if they get stuck later. The student handout (Supporting File S2. Leaf-cutter ants – Student handout) covers basic principles of image analysis and how to use ImageJ. Students can also watch three videos on specific image analysis tasks in ImageJ (Supporting Files S8. Leaf-cutter ants – ImageJ and regression videos, or consult the ImageJ user guide (https://imagej.nih.gov/ij/docs/guide/user-guide.pdf).

Students form groups and design studies

In a few minutes, students will work in groups to develop a research question and complete the rest of the lab. Since identifying a research hypothesis can be a point of difficulty, set some ground rules by giving a short lecture to the whole class before students form groups. First, hypotheses must be testable using data obtained from the images. Second, both the dependent and independent variables should be numerical. Once a group chooses one numerical variable to measure, they should choose another measurable numerical variable that they think might determine or influence the first variable. Explain that this constraint helps narrow down the many topics they may be interested in. Also explain that choosing to look at the relationship between two numerical variables constrains the type of statistical analyses needed to one approach, a simple linear regression. Explain that ordinarily, researchers follow their biological interests and use whatever statistical tools necessary.

Some instructors will want to carefully delineate correlation from simple linear regression (see [18] for more information about the differences between regression and correlation). We use "linear regression" as a descriptor throughout for simplicity here, while acknowledging the data, purpose of the analysis, student learning goals, and instructor philosophy might indicate one or the other as most appropriate.

At this point, students will work in groups to complete the lab. Assign students to groups of three to four. AF used responses to a student survey question ("How comfortable are you using Excel?") and performance on a previous Excel-based lab to form groups containing students with mixed levels of Excel proficiency. Ask students to introduce themselves to their group members and to share the research questions they brainstormed earlier. Ask students to exchange the lists of research questions they wrote earlier and read each other's ideas aloud.

Announce that groups will present a short (~4 slide) PowerPoint proposing their study mid-way though the lab period. Hand out a rubric (Supporting File S12. Leaf-cutter ants – Presentation Rubric) so students will know how final presentations will be graded. The proposal presentation should contain one slide for each project milestone: a research question, a hypothesis, proposed methods, and a figure or statement predicting the relationship between the independent and dependent variables. Groups will have the opportunity to revise their presentations in week two and add a results slide. Grade the final presentation using the rubric (Supporting File S12. Leaf-cutter ants – Presentation rubric). This low-key presentation is an opportunity for groups to get peer/instructor feedback before collecting data, and for students to learn from the strengths and weaknesses of their classmates' proposals.

Visit each group for 2-3 minutes to help groups develop their ideas and identify two numerical variables they would like to measure.

Ask groups to establish a common protocol every group member can follow to collect data. Each group should decide:

- How many ants they will measure (in total and per group member). You may wish to set a minimum sample size. On the other hand, a discussion could arise if two groups measure the same variables but choose different sample sizes yielding different results.
- How will they decide which images/videos to use (assuming they don't want to use all 98 photos and videos)?
- How they will ensure each ant is only represented once in the dataset (i.e., how they will avoid double-counting ants)?
- How they will operationalize their definitions? For example, ant body length could be measured "antenna to tail" or "nose to tail." Groups need a consistent approach to avoid measurement error.
- How far they will zoom in on each picture when setting the scale or measuring objects? Zooming in (to a point) reduces measurement error because it is easier to gauge where a measured line should start and end.

Check in with groups occasionally to ask guiding questions and confirm they are making progress. Encourage students to reference the handout and video tutorials to solve common ImageJ issues.

<u>Proposal presentations</u>

Reconvene as a class when all groups have finished their proposal PowerPoint presentations (or about halfway through the lab period). Allow each group to present, budgeting four minutes per presentation plus two minutes for peer/instructor feedback.

<u>Data collection</u>

When presentations are complete, groups are free to begin data collection. Most groups will need 40-60 minutes (the remainder of the lab period) to finish collecting data. Require group members to share their combined data with the instructor and each other before leaving lab. Spreadsheets hosted in the cloud (e.g., Google Sheets) can be an effective way to make sure everyone can access the data.

Week 2

<u>Background information: Linear regression</u>

Briefly recap week 1. Remind students they measured two numerical variables last week. Linear regression is one statistical approach for evaluating relationships between numerical variables. Introduce students to linear regression with a short PowerPoint or lecture. Describe the calculation and meaning of a linear regression, line of best fit, R2 values, and p-values, to whatever depth is appropriate for your course. There are descriptions of these concepts in the student handout (Supporting File S2. Leaf-cutter ants – Student handout). Additionally, there are many web resources that can be helpful to introduce students to linear regression, including videos we developed (Supporting File S8. Leaf-cutter ants – ImageJ and regression videos).

You can use the following question printed in the student handout as a quick knowledge check:

> *"Look at the data plotted [in scatterplot on page 5 of the student handout]. Here someone measured both the body length of 16 individual ants, and the area of the leaves they were carrying. Do you see a pattern here? How would you describe it in words?"*

Reinforce these concepts by asking students to complete the linear regression exercises (Supporting File S9. Leaf-cutter ants – Regression practice sheet). Students should complete the worksheet individually before discussing their answers with group members. Tell students they now have enough background information to analyze the data they collected last week.

<u>Creating scatterplots and running regression</u>

Over the next 40 minutes, groups should work together to create a figure of their data or visualization of their data, perform the regression analyses, interpret the results, and write a figure legend. The student handout links to instructions on how to run regressions in JMP, Excel, and R (Supporting Files S5, S6, and S7, respectively: Leaf-cutter ants – Conducting linear regression in Excel, Leaf-cutter ants – Conducting linear regression in JMP, and Leaf-cutter ants – Conducting linear regression in R).

At this stage, there is a danger that one group member will make the scatterplot or run the analysis while the others sit passively. Instructors should consider adding checks and structures to ensure that all group members are engaged in data visualization and analyses. For example, instructors could require students to complete anonymous peer evaluations, or to keep a log of each task they complete.

<u>Final presentations</u>

Ask groups to add one results slide to the PowerPoint they presented last week. This slide should include a figure and a figure legend (a short statement describing the main point of the figure and regression results). Check in with groups occasionally to ask guiding questions and confirm they are making progress. Reconvene as a class when all groups have finished their PowerPoint presentations. Facilitate presentations, budgeting five minutes per presentation plus two minutes for peer/instructor feedback.

TEACHING DISCUSSION

Implementation

Most students work through the image and regression analysis directions without extra help from the instructor. The common sticking points are three-fold:

- Identifying a testable research question,
- Planning a study that takes enough samples and collects the right information to address the research question,
- Interpreting the regression analysis in a meaningful way.

Stopping each group for a short discussion at each of those three points in the process is wise. Some groups may pose questions that are interesting but untestable given the constraints of the activity. Other groups may pick uninteresting questions because they are concrete or seem actionable. Try to respect students' ideas while steering them towards meaningful, testable hypotheses. Ask simple questions like "What information might [variable] tell you about leaf-cutter ant biology?" or "How could you extract information about [variable] from this photo?"

Questions like these will lead students to think about how they will proceed and what answers they are likely to find. Having students imagine (and even sketch graphically) their data before they collect it often heads off problems from the start. Students soon recognize if their proposed study will lead to a trivial answer or data that won't address their focal question.

When students learn new statistical concepts, they often focus on those concepts rather than the biology in their presentation of results. For example, a beginning student might emphasize the particular analysis and numerical result (e.g., "We conducted a linear regression and obtained a p-value of 0.0345."). A few reminders about the role of inferential statistics *as a tool* can help, as can providing examples of language emphasizing the biological result, with reference to the analyses only as supporting evidence (e.g., "Ants of larger body size, as measured by body length, carried leaf segments of greater area [leaf area (mm^2) = 0.3 * body length(mm) + 0.15; R^2 = 0.73, p = 0.019].").

Flexibility

This lesson is designed to be adaptable. Components of the lesson can be eliminated or augmented depending on time and students' background knowledge. For example, if students have experience with a variety of basic statistical analyses, this lesson could be repurposed to serve as a capstone assessment of data analysis skills. Students could choose numerical or categorical variables and then identify and apply appropriate analyses for their data. The post-lab reporting is also very flexible. Instead of giving presentations, students could write a complete lab report, or submit a scatterplot and short conclusion. We recognize instructors will need to carefully modify or supplement the regression teaching resources we provide to best meet the needs and prior experiences of their students. We suggest a few statistics resources that we find useful in Supporting File S4. Leaf-cutter ants – External resources.

This activity accommodates a variety of teaching styles. AF chose a relatively highly structured approach for this activity (e.g., by demonstrating how to take measurements in ImageJ in the front of the class). Instructors could also choose a less structured approach. When JW teaches this lesson, he spends very little time presenting in front of the whole class. Instead, students work at their own pace using the detailed handouts and videos as guides. Letting students work through the instructions frees the instructor to spend more time working with individual groups on their areas of concern or need. Only occasionally are there student logistical questions or problems that cannot be answered by the handout or video. The benefit is the ability to accommodate different group paces, where students receive the instruction they need when they need it. One cost is the loss of exposure to the ideas and questions of other groups. Using this approach, most student groups can graph their data and conduct linear regression during a three hour lab period.

The videos and instructions for image analysis steps, regression concepts, and regression calculation steps are generalizable, and could be repurposed for other lab or field studies. Students who perform image analysis in our courses often remember it and offer it as a useful tool to measure other biological features in later coursework.

Dividing up the work

There are too many images and videos for one student to measure in a short period of time. How many samples to do and how many questions should be addressed is therefore a function of class/group size, available time, and instructor preference. Letting groups choose their own question provides more student ownership, but requires the instructor to talk to each group about their proposed work. Another option would be to have a class discussion and vote on a consensus hypothesis and study design. That approach would allow for measuring all images, with the statistical power that larger sample provides.

Emergent opportunities

This activity sparks productive conversations about experimental design. Sometimes groups ask similar questions but get different answers. For example, two groups in AF's class found opposite relationships between ant head length and leaf length. These groups chose very different sample sizes, leading to a good discussion about statistical power. As another example, several groups in one of JW's classes independently decided to investigate the relationship between leaf size and ant body size, as it is one of the most obvious questions to ask. However, different groups decided to measure leaf and ant size by different metrics, and got surprisingly different answers. A group that looked at leaf area versus ant length did not find a relationship. Another group that looked at leaf area versus ant cross-sectional area found a strong, positive relationship. This discovery led to a great impromptu discussion about how we define concepts of interest, and how we can or should generalize from any individual study.

Student feedback and learning

In 2013, we did some preliminary assessments of student perceptions and learning of quantitative skills. Of 37 students surveyed, 84% agreed or strongly agreed that the biological context of leaf-cutter ant foraging made learning statistics more interesting. 92% agreed or strongly agreed that image analysis was a useful general skill for biology students to learn. On average, there was a 15% increase in scores on a short assessment ($p<0.001$, paired t-test comparing pre- to post-test student performance). This is a modest improvement, but still encouraging after only a single class experience with a new topic that requires some nuanced understanding. Greater learning gains are likely with reinforcement, practice, and follow-up assignments.

Since then, the lesson has been evaluated formally or informally by seven faculty at four institutions, and refined based on their feedback. In particular, several faculty participated in a QUBES (Quantitative Undergraduate Biology Education and Synthesis: https://qubeshub.org) Faculty Mentoring Network where they explored and implemented one of four lessons that use image analysis to motivate student learning of quantitative skills (of which this lesson was one option - see https://aimslabs.org for others). Faculty commented on the ease with which students get up to speed with image analysis and the biological context, and how smoothly the lesson seems to go. One instructor put it simply: "Students really enjoyed the activity, and I would definitely use it again in a future course." When surveyed, the majority of students agreed or strongly agreed that the lesson increased their interest in learning statistics (Figure 1).

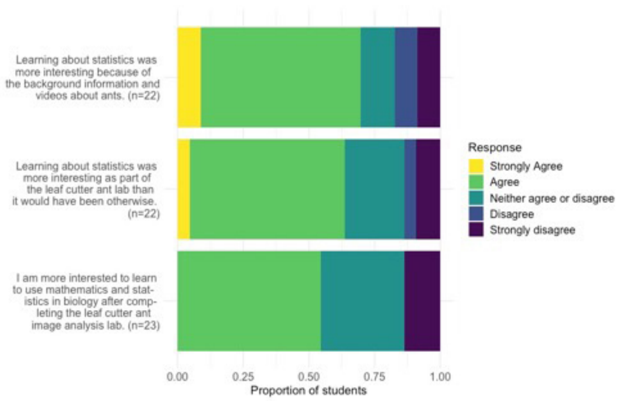

Figure 1. Results from student perception surveys at a private four-year college where the lesson was expanded to a larger multi-week project with greater emphasis on background literature research.

SUPPORTING MATERIALS

- S1. Leaf-cutter ants – Alternative teaching strategies
- S2. Leaf-cutter ants – Student handout
- S3. Leaf-cutter ants – Introduction video. Video credit: Justin Touchon, Myra Hughey, and Jeremy Wojdak.
- S4. Leaf-cutter ants – External resources
- S5. Leaf-cutter ants – Conducting linear regression in Excel
- S6. Leaf-cutter ants – Conducting linear regression in JMP
- S7. Leaf-cutter ants – Conducting linear regression in R
- S8. Leaf-cutter ants – ImageJ and regression videos. Video credit: Daniel Metz.
- S9. Leaf-cutter ants – Regression practice sheet
- S10. Leaf-cutter ants – Regression assessment
- S11. Leaf-cutter ants – Instructor slides
- S12. Leaf-cutter ants – Presentation rubric
- S13. Leaf-cutter ants – Alignment with AP Biology, Common Core, and NGSS standards

ACKNOWLEDGMENTS

We acknowledge the National Science Foundation Improving Undergraduate STEM Education (IUSE) program for funding the AIMS: Analyzing Images to learn Mathematics and Statistics project through grant DUE-IUSE 1431671. Arietta Fleming-Davies, Nicole Chodkowski, Jennifer Hanselman, Emma Perry, and Patricia Saunders tested this lesson in their classrooms and provided valuable student responses and formative feedback on the materials and instructional approaches. Participant responses covered by Radford University IRB protocol 16-059.

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Table 1. Leaf-cutter ant lesson teaching timeline

Activity	Description	Time	Notes
Preparation for Class			
Review student handout	Make one copy of student handout for each student.	5 minutes	The student handout is available in the Supporting Files section (S2).
Practice using ImageJ	Download ImageJ. Practice opening photos in ImageJ, zooming in and out, setting a scale, measuring straight lines, measuring polygons, and viewing the measurement results.	15 minutes	ImageJ is a free image analysis software available to download here: https://ImageJ.nih.gov/ij/
Load Data Analysis ToolPak	Load the Excel Data Analysis ToolPak if you plan to use Excel to run regression.	5 minutes	The Data Analysis ToolPak is a free, menu-based statistics add-in. For instructions on downloading the ToolPak, consult online help resources for the version of Excel your students will use.
Assign pre-lab reading	To prepare for the activity, ask students to read the student handout (S2) and watch the leaf-cutter ant introductory video (S3).		The student handout and video are available in the Supporting Files section (S2 and S3, respectively).
Lab Session 1 (~3 hours)			
Mini-lecture: Leaf-cutter ants	Background information on leaf-cutter ant biology.	20 minutes	Short reading under the "Leaf-cutter ant foraging" section of the student handout. The leaf-cutter ant video (duration = 2:30 minutes) is available in the Supporting Materials section (S3).
Mini-lecture: Introduction to image analysis	Background information on image analysis concepts.	5 minutes	Background on ImageJ is available on pages 2-4 of the student handout (S2).
Activity: Practice using ImageJ	Open ImageJ. Open a photo in ImageJ. Practice drawing lines using the Straight Line tool. Practice setting the scale. Practice making and recording measurements using the Straight Line tool. Practice making and recording measurements using the Polygon tool.	15 minutes	There is a link to a practice image on page 3 of the student handout. Additional instructions on using ImageJ are available on page 3 of the student handout (S2).
Plan study	Form groups, develop research questions, plan workflow, create short proposal presentations.	30 minutes	Guidance on hypothesis testing is available on page 4 of the student handout.
Proposal presentations	Groups present research proposal presentations.	30 minutes	
Collect data	Groups complete data collection.	60 minutes	Groups should share data with group members and the instructor before leaving lab.
Lab Session 2 (~3 hours)			
Mini-lecture	Restate purpose of module, overview regression analysis.	30 minutes	Background on linear regression is available on page 5 of the student handout (S2).
Activity: Linear regression exercise	Students complete Regression Practice Sheet individually, then discuss answers within groups.	30 minutes	The linear regression exercise is available in the Supporting Materials section (S13).
Visualize data and run regression	Create figures, run regression, interpret results and write figure legends.	40 minutes	Ask students to actively contribute and log specific tasks completed. Group members should review and sign each other's task logs. Logs should be submitted at the end of lab.
Create final presentation	Groups add a results slide with a scatterplot and figure legend to the presentation they created last week.	20 minutes	
Present final presentation	Groups present their findings. budgeting five minutes per presentation plus two minutes for peer/instructor feedback.	60 minutes	