BCEENET
BIOLOGICAL COLLECTIONS IN ECOLOGY AND EVOLUTION NETWORK

Teaching Notes- Sexual Dimorphism CURE: Exploring Melanized Wing Patterns of Pieridae Butterflies

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CURE SUMMARY

Sexual selection has been shown to play an important role in the evolution of wing patterns in butterflies, Melanization is also used for thermoregulation, and can be seasonally plastic in Pieridae butterflies. *Pieris rapae*, the cabbage white butterfly, is an invasive species and is abundant in natural history collections. In this Course-based Undergraduate Research Experience (CURE), students will explore variation in sexually dimorphic wing patterns of Pieridae butterflies using digitized natural history collections (dNHC) data. Students will learn how to organize and clean digital collections data, navigate data portals for information, use mapping software to demonstrate species distributions, and use image analysis software to measure phenotypes. You will also have the opportunity to participate in a cross-institutional research project, contributing to and analyzing a shared dataset.

Intended Audience: Undergraduate, all levels. Suitable for in-person, hybrid, or online courses in biology or environmental science.

Learning Time: Flexible, from 5-15 weeks

Required Resources: Access to computers and internet. All resources, data, and tools needed to complete this CURE are freely available online (iDigBio, GEOLocate, ImageJ, QGIS). No physical lab or additional materials are needed.

Optional Tools: R Studio (also free and open-source)

STUDENT LEARNING OBJECTIVES

- Locate, evaluate, and interpret relevant scientific literature
- Formulate and test hypotheses
- Communicate using evidence-based arguments and background research
- Download and clean dNHC data
- Accurately make and record measurements, including organizing and annotating datasets
- Identify methodological problems and troubleshoot them
- Analyze morphometric data with appropriate quantitative methods
- Analyze spatial data and generate maps that effectively communicate research results
- Work effectively as part of a collaborative group
- Recognize and evaluate increases in your ability to do scientific research
- Develop a sense of belonging in the scientific community

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DESCRIPTIONS OF ACTIVITIES AND ASSESSMENTS

Students will utilize the data portal iDigBio to access specimen data of Pieridae butterflies, including collection localities, dates, and digital images. They will download, organize, and clean the specimen data, and improve the dataset through georeferencing and morphological measurements on digital images. The resulting dataset will then be used to answer research questions on sexual and natural selection. Formative assessments will include exercises and worksheets, as well as intermediate data products (e.g., morphological data). As a summative assessment, students will produce a final product (research paper, poster, and/or presentation) that will draw conclusions from their data.

CURRICULUM MATERIALS PROVIDED

**Core materials shared across all BCEENET CUREs*

- Scientific Background on Melanization in Pieridae Butterflies
- Annotated Bibliography- Sexual Dimorphism CURE
- Synthesis Matrix Assignment
- Data Management Activity
- iDigBio Search Activity
- *Measuring Size and Area of Digitized Specimens using ImageJ (Sorojsrisom, Krumm, & Trillo, 2022)
- Repeatability Exercise for Morphometric Measurements- Student Handout
- Repeatability Exercise- Data collection spreadsheet for R and other statistical packages
- Repeatability Exercise- R script (RepeatabilityScript.R)
- Repeatability Exercise- Data collection spreadsheet for ANOVA in Excel
- *Putting Specimens on the Map: An Introduction to Georeferencing (Sorojsrisom & Johnson, 2022)
- *Mapping Specimen Occurrence Data with QGIS (Bronson, 2021)
- CURE Self-Assessment #1
- CURE Self-Assessment #2
- CURE Self-Assessment #3
- Group Management Agreement
- Group Assessment Handout
- Final Group Assessment Handout

STATEMENT ON EQUITY AND INCLUSION

This undergraduate research experience is designed to be accessible to all students enrolled in the CURE course, regardless of their ability to attend classes on campus. Its flexible lesson-plan design provides both asynchronous and synchronous options for most research activities. Students will need access to only the internet and a computer to complete the assignments.

We recommend you include a statement in your syllabus stating: In this class we will all work to create and support a culture of belonging where every participant in the research experience is recognized and respected for their individual contributions to the success of the project. We will treat each other with mutual respect and tolerance during all interactions, making the classroom into a safe collaborative space for all individuals.

Instructors should also be aware of the racism and colonialism that is an important part of the history of natural history collections (Das & Lowe, 2018; Ashby, 2020). You may consider incorporating readings on this topic into your course. iDigBio has A Diversity, Equity, and Inclusion Reading List, with Special Emphasis on Natural Sciences and Natural History Museums that we highly recommend. Here are two readings that fit well with this CURE:

> Ashby, J. (2020). Telling the truth about who really collected the "Hero Collections". Blog of Natural Sciences Collection Association. https://natsca.blog/2020/10/22/telling-the-truth-about-who-really-collected-the-hero-collections/
>
> Das, S., & Lowe, M. (2018). Nature read in Black and White: Decolonial approaches to interpreting natural history collections. *Journal of Natural Science Collections 6*: 4-14. http://www.natsca.org/article/2509

IMPLEMENTATION TIMELINE

Overview

	Topics	**Activities & Assessments**
Week 1	Introduction to the CURE	Complete CURE Self-Assessment #1
Week 2	Introduction to *Pieris rapae* and sexual selection	Review research paper on melanization patterns in *Pieris rapae*; begin Synthesis Matrix Assignment
Week 3	Downloading and managing collections data from iDigBio	Complete Data Management Activity; complete iDigBio Search Activity and submit downloaded datafile and metadata file; form student research groups and complete Group Management Agreement
Week 4	Data management- Cleaning dates	Create and submit research questions and hypotheses; submit dataset with cleaned dates and updated metadata file; upload ImageJ on student computers (if appropriate for your course)

Week 5	Collecting morphological data- Establishing repeatability	Walkthrough ImageJ instructions and troubleshoot methods as a group activity; complete Repeatability Assignment
Week 6	Collecting morphological data- Generating new measurements	Collect and submit morphological data on assigned specimens; update metadata file; first Group Assessment due (deadline variable, based on course schedule); peer-review student data (optional, but recommended)
Week 7	Introduction to georeferencing	Complete georeferencing exercise; clean and improve georeferencing of collections sites for assigned specimens; update metadata file; complete CURE Self-Assessment #2
Week 8	Introduction to mapping	Download and install QGIS on student computers (if appropriate for your course); complete QGIS exercise; second Group Assessment Due (deadline variable, based on course schedule)
Week 9	Mapping workshop	Create maps using student data relevant to student research questions
Week 10	Workshopping and troubleshooting	Complete any remaining data collection, cleaning, and mapping with support from instructor
Week 11	Spatial analysis and data analysis	Complete data analysis plans and discuss potential sources of error and the limitation of collections datasets; third Group Assessment due (deadline variable, based on course schedule)
Week 12	Data analysis workshopping	Complete data analyses and finalize all figures, tables, and maps needed for summative assessments (posters, papers, infographics, oral presentations)
Week 13	Summative assessment workshopping	Workshop summative assessments; complete any review of drafts and ask final questions
Week 14	Summative assessment workshopping	Complete summative assessments (posters, papers, infographics, oral presentations); complete CURE Self-Assessment #3; complete Final Group Assessment

Detailed Timeline

Week 1: Introduction to the CURE

- Provide a broad overview of the Sexual Dimorphism CURE
    a. Introduce the CURE
    b. Review research activities
    c. Highlight collaborative, multi-institutional nature of this project
- Introduce natural history collections as a research resource
    a. What are collections and where are they found?
    b. How are collections used for research?
    c. What are advantages and challenges of using collections data?
- Ask students to reflect on their experience and confidence in research

Materials Needed

- CURE Self-Assessment #1- *Additional information on the Self-Assessments can be found in the* ***Implementation Notes*** *section at the end of this document.*

Assessments

- CURE Self-Assessment due

Potential Add-ons

- Helpful videos for students or in class
    - Pieris video (most relevant up to 1:05)
    - Collections video- 33 Million Things, from the American Museum of Natural History

Week 2: Introduction to Pieris rapae and sexual selection

- Introduce *Pieris rapae* melanization
- Review a research paper about the impact of sexual selection and thermoregulation on melanization patterns in *P. rapae* (see annotated bibliography provided)
- Assign students additional research papers to review and complete the Synthesis Matrix Assignment (individually or in groups)

Materials Needed

- Scientific Background on Melanization in Pieridae Butterflies (for students)
- Annotated bibliography- Sexual Dimorphism CURE (for instructors)
- Synthesis Matrix Assignment

Assessments

- Synthesis Matrix Assignment

Potential Add-ons

- Ask student groups to present results from a figure in the research paper

Week 3: Downloading and managing collections data from iDigBio

- Introduce digitized natural history collections data and the iDigBio data portal
- Download *Pieris rapae* datasets from iDigBio
- Discuss contents of the iDigBio dataset
- Introduce basic data management skills, including creating a metadata file

Materials Needed

- iDigBio Project Overview video
- Data Management
- iDigBio Search Activity
- Data Organization in Spreadsheets for Ecologists from Data Carpentries (Instructor resource)
- Group Management Agreement- *Additional information can be found in the* ***Implementation Notes*** *section at the end of this document.*

Assessments

- Data Management Activity
- Downloaded datafile
- Metadata file
- Group Management Agreement

Potential Add-ons or Modifications

- Students read and discuss Broman & Woo (2018), an article on data management and organization in spreadsheets.

Week 4: Creating hypotheses & data management- Cleaning dates

- Create research questions and hypotheses. We recommend creating a single class-wide research question and a secondary research question for each student group. Each student research group would ultimately be responsible for analyzing the data to answer both their class and group research questions.
- If you are participating in the collaborative collection of data and would like access to the shared dataset, please contact bceenetwork@gmail.com. Give students the shared data file this week.

  OR

  If you are NOT participating in the collaborative collection of data, have students use the *Pieris rapae* data file downloaded from iDigBio during week 3.
- Assign specimen records to individual students or teams. We recommended 5-10 records per student.
- Review the data management of dates and use of Julian date calendars
- Instruct students to clean dates for their assigned specimen records
- Assign the students to upload ImageJ on their personal laptops if they are not using computers provided by the institution.

Materials Needed

- Class dataset
- Julian date calendar

Assessments

- Research questions and hypotheses
- Dataset with cleaned dates
- Updated metadata file

Potential Add-ons or Modifications

- If you did the annotated bibliography add-on, students could present one of the research papers they examined, either independently or in groups.
- If your course has a large writing component, the submission of research questions and hypotheses can be expanded into a grant proposal assignment

Week 5: Collecting morphological data- Establishing repeatability

- Walk through *Measuring Size and Area of Digitized Specimens using ImageJ* (Sorojsrisom, Krumm, & Trillo, 2022) with your students. Students will need lots of time and repeated instructions. Expect to spend a significant amount of the class period on troubleshooting. There is an assignment associated with this activity.
- Instruct students how to practice collecting data through the repeatability exercise. They will need to complete this exercise with a high level of repeatability before they collect data for analysis. **Note- If you are participating in collaborative data sharing, your students MUST complete this activity.**

Materials Needed

- *Measuring Size and Area of Digitized Specimens using ImageJ* (Sorojsrisom, Krumm, & Trillo, 2022)
- Repeatability Exercise for Morphometric Measurements Student Handout- *Additional information can be found in the **Implementation Notes** section at the end of this document.*
- Repeatability Exercise- Data collection spreadsheet for R and other statistical packages **OR** Data collection spreadsheet for ANOVA in Excel
- Repeatability Exercise- R code (optional)

Assessments

- Activity from *Measuring Size and Area of Digitized Specimens using ImageJ* (Sorojsrisom, Krumm, & Trillo, 2022)

Potential Add-ons or Modifications

- If you want to adjust the amount of time your students dedicate to morphology measurements, change the number of specimens assigned to each student.

Week 6: Collecting morphological data- Generating new measurements

- Students will continue to work on morphology measurements in class with support from their instructor.
- If some specimens cannot be measured, they can be swapped for other specimen records. *Your class is likely to encounter a few specimens with images of the data label only, the ventral surface of the butterfly, or damaged specimens that cannot be measured.*
- When students complete their measurements, the instructor should review the data for outliers and problems. Students can be asked to remeasure problematic specimens.

Materials Needed

- *Measuring Size and Area of Digitized Specimens using ImageJ* (Sorojsrisom, Krumm, & Trillo, 2022) for reference

Assessments

- Dataset with morphological data due to instructor for review (deadline can be extended back several weeks if needed for completion of assignment and/or remeasuring of specimens)
- Updated metadata file
- First group assessment due (the timing and number of group assessments will depend on your course schedule and number of students per research group)

Potential Add-ons or Modifications

- Several instructors have had great success with running a peer review process for the data collected.

Week 7: Introduction to Georeferencing

- Students are introduced to georeferencing using GeoLocate and Google Maps, learning to determine latitude and longitude, and uncertainty.
- Students will georeference all of their assigned specimens, either confirming and/or correcting current georeferences or adding new georeferences.

Materials Needed

- *Putting specimens on the map: An introduction to georeferencing* (Sorojsrison & Johnson, 2022). Materials include student handout, georeferencing exercise, and georeferencing video

Assessments

- Georeferencing exercise from *Putting specimens on the map: An introduction to georeferencing* (Sorojsrison & Johnson, 2022)
- Dataset with georeferenced localities
- Updated metadata file
- CURE Self-Assessment #2 due

Potential Add-ons or Modifications

- If you wish to reduce the number of weeks dedicated to the CURE, the majority of the *Pieris* specimens already have latitude and longitude provided and can be mapped without additional georeferencing.

Week 8: Introduction to Mapping

- Students are introduced to mapping using QGIS and create practice maps.

Materials Needed

- QGIS- Download onto student or institutional computers. Be sure students download the **Long term release (Most stable)**!
- *Mapping Specimen Occurrence Data in QGIS* (Bronson, 2021)

Assessments

- Mapping exercise from *Mapping Specimen Occurrence Data in QGIS* (Bronson, 2021)
- Second group assessment due (the timing and number of group assessments will depend on your course schedule and number of students per research group)

Potential Add-ons or Modifications

- Basic mapping can be completed online with Google Earth if you are unable to provide access to QGIS for your students.

Week 9: Mapping workshop

- Students create maps of the Week 8 georeferenced specimen records using QGIS.

Materials Needed

- QGIS downloaded onto student or institutional computers
- *Mapping Specimen Occurrence Data in QGIS* (Bronson, 2021) (for reference)

Assessments

- Map of georeferenced specimens

Potential Add-ons or Modifications

- Students can map their group georeference data or the combined georeference data for the entire class

Week 10: Workshopping and Troubleshooting

- Students complete any final data collection with support from instructor

Materials Needed

- No new materials needed

Assessments

- Final datasets due

Week 11: Spatial analysis and data analysis planning

- Student teams develop data analysis plans, including specific plans for all tables, figures, maps, and statistical analyses needed to answer their research questions. This can be supported through entire class brainstorming and discussion or discussions with individual teams in smaller courses. Allot time for meeting with individual students or student teams to discuss their plans.
- Data analysis can include any combination of graphs, maps, and statistical tests depending on the level of your students and your course learning objectives.
- Students brainstorm and discuss potential sources of error and the limitations of collections datasets.

Materials Needed

- Scientific Background on Melanization in Pieridae Butterflies (for reference)
- Previous student submissions of Synthesis Matrix Assignments and group questions and hypotheses (for reference)

Assessments

- Third Group Assessment due (the timing and number of group assessments will depend on your course schedule and number of students per research group)
- Research team data analysis plans for instructor review

Potential Add-ons or Modifications

- Instructors have successfully used a wide variety of statistical tests, statistical programs (R studio to Excel), and other analyses for this step.

Week 12: Data analysis workshopping

- Students incorporate feedback from instructors to finalize their data analysis plans
- Students complete their data analysis, creating all figures, tables, and maps needed for their summative assessment (posters, papers, infographics, oral presentations)

Materials Needed

- QGIS
- Statistical package (i.e., R) or other program chosen by the instructor (i.e., Excel)

Assessments

- Student groups submit all figures, tables, and maps for review

Week 13: Summative assessment workshopping

- Students work on summative assessments. These assessments may include posters, papers, infographics, or other assessment of the instructor's choosing.

Materials Needed

- No new materials needed. Access to QGIS and statistical packages may be helpful.

Potential Add-ons or Modifications

- If teams are giving both an oral and written assessment, this may be a good week for the oral assessment. Rough drafts might also be reviewed at this time.

Week 14: Summative assessment workshopping

- Students complete summative assessments. Any final help needed and review of drafts would be completed at this time.

Materials Needed

- No new materials needed. Access to QGIS and statistical packages may be helpful.

Assessments

- Final summative assessment due
- Final Group Assessment due
- CURE Self-Assessment #3 due

IMPLEMENTATION NOTES

Repeatability exercise for morphometric measurements

In this activity, students will learn how to measure repeatability for the morphological measures they conduct with image J. Through a written tutorial and optional guided lecture, students will then learn how to analyze their measurements and indicate the level of repeatability.

- Repeatability measures variation that occurs when conditions are constant, and the measures are collected by the same person within a short amount of time. Therefore, all measures used for this exercise will need to be done within a short period of time.
- Depending on the level of the course, instructors can use an R script (example included) and ANOVA test to calculate repeatability coefficients (r), or they can calculate the standard error and use it as a proxy for repeatability. Both options are included in the exercise.
- Important: While repeatability coefficients (r) have a general criterion for what is a low versus a high repeatability, standard error is used as a proxy. This means that there is no pre-determined criterion for what is a high or a low standard error value in terms of repeatability. Often several values are compared to each other, and the lower the standard error (up to 0), the lower the variation among the measurements. However, the instructor and students will need to determine whether the variance is low enough to meet their standards.

Group Management Agreement

The **Group Management Agreement** document is meant to help your students assign responsibilities and self-manage their groups. This is an important component of scientific research, which is often collaborative and involves members with different backgrounds, skills, and comfort levels regarding different tasks.

This document should be assigned fairly early in the course, after some introductory material and after groups have been formed, but before group work commences. The students will sign up to be the 'lead' on certain assignments, meaning they will take responsibility for overseeing the group work that is done on that assignment. Please note that this means that this means they are responsible for organizing the group work for this assignment and making it clear who is responsible for which parts, checking group members' progress, collecting all the group work together, and turning it in on behalf of the group by the due date for that assignment - they are not signing up to perform all the work for that assignment by themselves. It is okay that they may not yet be familiar with what each assignment entails - this is part of the learning process as well. The important thing is that the students divide leadership for assignments equally amongst themselves and understand that they are the 'lead' member in charge of the assignment, but that all their group members are expected to contribute equally to each assignment.

Most courses implementing this CURE require that each student perform all the tasks (metadata, georeferencing, morphometrics) for 10 specimens, and that the students divide up the rest of the work (e.g. analysis, mapping, background research, presentations and final paper) equally. However, you may opt to adjust this format by allowing students to divide up the work in different ways (e.g. two students may do all the morphometric measurements, two others do the background research, etc.). *This can increase the accessibility of your course if not all students have access to the computer resources necessary for all tasks.* Make sure the way you want your students to divide up the work between them is clear, and if you opt for a more free-form approach, it may be a good idea to add another table to the Group Management Agreement that asks the students to explicitly state who is responsible for which assignments.

Self Assessment

The **Self Assessment** assignment is intended to help students reflect on their growth throughout the semester and give you an idea of what they are thinking. Simply completing the assessment serves as a reflection exercise that encourages students to think about their own learning.

The assessment should be assigned 3x throughout the semester: Once at the beginning of the course, once in the middle, and once at the end. There are three different documents for each of these time points, as the questions change a bit each time. This assignment can be used as "easy points" for your students. We suggest a simple completion grade. The mid-semester assessment may be particularly helpful as a check-in, as you will be able to assess how your students are doing and assist any students that may be struggling.

REFERENCES

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