HOW-TO-DO-IT

Team Echinacea & Construction of a Key Using Online Images of Fresh Prairie Plant Pollen

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Abstract

In the summer of 2009, as an RET (Research Experience for Teachers), I joined the research team studying Echinacea angustifolia by assisting with ongoing research. I also prepared botanical collections for my classroom. These collections consist of flowering botanical specimens and prepared slides of fresh pollen from each plant. Digital images of fresh pollen from these plants were captured to build an online digital pollen library. A portion of the online pollen images serves as an original dichotomous key to be constructed by students in 10th grade Biology. This article describes the field activities of the research team and the process for the plant and pollen collection as well as instructions for the pollen lesson implementation. The national content standards (9–12) for history and nature of science involved are G.1: "Develop an understanding of science as a human endeavor" and G.2: "Develop an understanding of the nature of scientific knowledge."

Key Words: Inquiry; dichotomous key; pollen; Echinacea angustifolia.

O Team Echinacea

Since 1995, *Echinacea angustifolia* plants have been studied on prairie remnants in Douglas County, Minnesota. Two gardens of plants at 1-m intervals also serve as sites. Team Echinacea consists of multiple researchers on several projects. Undergraduate students seeking field experience spent most of their time in data collection, with a portion of time on a self-directed project. As an RET participant, my role was similar to that of the undergraduates, but I was given more latitude to assist other

projects and freed from weekend data collection. The graduate students and postgraduate students spent equal amounts of time contributing to data collection and working specifically on their projects. The research team leaders also participated daily in data collection. As the various collection protocols were developed by the lead researchers, it was helpful for them to be present to instruct others in the group. They also refined many of the protocols for easier learning, recording, and data analysis.

The major projects ongoing with *E. angustifolia* involve conducting seedling searches, documenting phenology, and measuring plants (Wagenius, 2004, 2006). The data for the seedling searches were plotted according to locations around known flowering plants with a 1-m search diameter (Figures 1 & 2). The plant locations were measured

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and marked using surveying equipment and matching an existing map. Seedling survival can be tracked on a yearly basis, especially as a plant matures toward its flowering. As a personal note, spending several hours a day on hands and knees searching for a small dicotyledon seedling truly provides a "field experience."

The second two data collections involved entering information from individually tagged, numbered specimens into prepared forms on a handheld "personal digital assistant" (PDA). The PDA data were transferred to a "master" file that holds all the compiled data for the day and field season. This process allowed from 2 to 10 people to collect data during a session, maximizing effective times for the group to work. The phenology data collection involved identifying the first day of flowering, length of flowering time, and last day of flowering. *Echinacea angustifolia* is a composite flower that produces individual florets each day. It releases

> female structures one day and male structures the next, which allows first and last days of flowering to be identified without checking each plant daily (Figure 3). Phenology was documented on alternate days from the day of first flowering in the common garden site and at Staffanson Prairie Preserve (a Nature Conservancy site) until the last (14 July to 19 August).

> The most complex data collection was "measuring" of the gardens. The measuring dealt with the height of the plant, whether it flowered or was basal, how many heads flowered, and any damage on the plant caused by various insects. With just over 10,000 plants, the measuring work needed

to be shared by the team. Working in pairs, one entered into the PDA the data from the partner as the other measured, counted, and identified according to the protocol. This was a tedious, demanding task as both people were either kneeling or bent over the plant to be measured. The combination of weather and insects added to the challenge.

○ Species Collection

In addition to the main research projects, I was able to work on my collections. The flowering plants in my collection were those that flowered at the same general time as *Echinacea angustifolia*. With some practice and guidance from other members of Team Echinacea, I was able to

The American Biology Teacher, Vol. 73, No. 1, pages 35–38. ISSN 0002-7685, electronic ISSN 1938–4211. ©2011 by National Association of Biology Teachers. All rights reserved. Request permission to photocopy or reproduce article content at the University of California Press's Rights and Permissions Web site at www.ucpressjournals.com/reprintinfo.asp. DOI: 10.1525/abt.2010.73.1.7



Figure 1. *Echinacea angustifolia* seedling (near pencils; picture by author).



Figure 2. Searching for seedlings in a 1-m² quadrat (picture by author).



Figure 3. *Echinacea angustifolia* head with perimeter florets flowering. The pollinator *Agapostemon texanus* is visiting the perimeter florets to take pollen. A second head is also in view that hasn't reached the flowering stage (picture by author).

comfortably identify many native plants. Throughout the summer, I started to get a sense of the balance among cool-season grasses, legumes, forbs, and warm-season grasses. The focus on E. angustifolia kept my collection to legumes and forbs as coflowering species. I collected plants that displayed fresh pollen, harvested the pollen, and dried the plant to keep as a preserved specimen. In order to have fresh pollen, no pollinators could have visited the flower prior to the collection. This process required careful selection of suitable pollen-presenting plants early in the flowering day. Some pollen could be shaken off anthers while held with a forceps; others had to be partially dissected so that the anther could be exposed enough to remove the pollen. The harvested pollen was transferred to a microscope slide onto a drop of glycerin (which preserved the pollen but did not cause it to swell or shrink because of water loss), and a cover-slip was placed over the pollen on the drop. Three slides were made for each species. Each slide was viewed to verify that it had pollen and that the three slides from a species were the same. Digital images were captured at this stage, when the pollen was as fresh as possible. Between plants, the forceps and other equipment were cleaned and the surfaces wiped to minimize cross-contamination with other pollen. The cover-slips were adhered to the slides and left to cure and be labeled for the collection.

I believe the collection and preparation of a wet-mount slide of pollen to be a useful practice for biology students. Pollen may be easier to deal with than microorganisms, as it is not motile and most samples have a natural pigment that makes them stand out. The collection of plants as herbarium samples is another lab skill that can be introduced in class. Both skills can be practiced on garden plants – especially annuals toward the end of the growing season when school is in session. For Team Echinacea and especially for me, the surprise of what each plant's pollen looked like was a scientific thrill.

• Experimental Design

While field work held physical demands, the hardest work was the experimental design process. Before any of the individual projects of Team Echinacea began, they needed to be planned for length, sample size, and data collection protocol. While one measures and collects, the awareness of the overall experiment's design is kept in mind. The protocol always dictates the process, and the data collection process must always be aligned to the goals of the research. (While measuring height, for instance, there is always "competition" for the tallest plant, both because the data are important to the project and because it gives some bragging rights to the person who measures it on that day).

Researchers must take steps to preserve the integrity of their data while knowing that the information may prove or disprove their test. Random sampling was frequently employed with this aim. Whether plant selection, time selection, or choosing which human collected the data, everything possible was randomized to some degree. Even when the data were qualitative in nature, they were subjected to statistical analysis after collection. This is something I fully expected with quantitative data, but the structure of the experiment allowed for any type of data to be processed numerically. Designing experiments is a process skill that is learned with repetition. Consuming experimental results by reading, discussing, or simulating can be a faster process than repeating the experiment. Producing an experimental design takes more time, but students can put their skill knowledge into practice. The practice of designing experiments and consuming experiments both improve students' understanding of the inquiry process.

O Lesson Instruction & Implementation

Discovery drives the field of biology, but we cannot discover everything a second time. Students generally have not seen pollen or images of pollen prior to high school. Whether native or cultivated, the pollen for specific plants waits to be discovered. I wanted to transfer that spirit of discovery





Figure 4. *Carduus nutans* (nodding plumeless thistle, 400×; picture by author).



Figure 5. *Amorpha canescens* (leadplant, 400×; picture by author).

of pollen types into my classroom. The lesson deals with pollen that students likely have never seen. The image samples are purposely unlabeled to keep them from associating similar species by similar family name. The lesson also gives them the sense of being in uncharted territory.

The lesson developed from an assignment in an online hybrid course from Black Hills State University in Spearfish, South Dakota. The requirements were to follow the 5E Learning Cycle model for lesson instruction (Llewellyn, 2004). Following the 5E Learning Cycle to plan a lesson forced me to structure each phase of the lesson to be less teacher-directed and more student-directed. Another requirement was to involve the Reformed Teaching Observation Protocol (RTOP) developed at Arizona State University. The rubric is normally used as a postlesson evaluation of an inquiry lesson. The RTOP was used as a guide to construct the lesson to be inquiry-based. The existing pollen key from Team Echinacea provided an opportunity to meld these elements together.

The national content standards (9–12) for science as inquiry that were met were A.1.f: "Communicate and defend a scientific argument"; and A.2.f: "Understanding about scientific inquiry: Results of scientific inquiry – new knowledge and methods – emerge from different types of investigations and public communication among scientists."

Engagement

The students will look at microscope-slide pollen samples. These can be prepared slides or student-made slides. If the students have prepared the slides as part of a lab process, this stage may occur as part of closure to that exercise and engagement with the pollen key. Any pollen that appears as colored dust should work well as a sample. Most flowers that present pollen for insect pollinators on visible or raised anthers are recommended. The only caution would be that some students may experience allergies to certain pollen (i.e., ragweed or tree pollen).

The students can respond to the following questions: *Is all pollen the same? What are some differences expected among pollens? Will plants with similar pollen have other similar characteristics?* My expectation was that students would anticipate that there are different pollen types. I expected that they would know that different pollens are transported by different means (air, insects, and animals).

Exploration

Observing, classifying, and communicating are the three process skills of focus for this lesson. Most of the pollen samples have distinguishable exterior surfaces; some have coloration and size differences. Most of the keys will draw on these observations. The classifying process will come from separation of pollen samples by the observed characteristics. My 10th-grade students had previously used dichotomous keys and have constructed an original key of 20 insects. The pollen samples differ from organisms used in other keys in that students likely will not recognize any of the pollen types, whereas students already know many common insect names and identifying characteristics.

It is during this process that the communication skill is most necessary. Students may confer with one another to classify or key the pollen grains. At this stage begins the process of cross-checking from one student to another. This dialogue between students can involve comparison of characteristics to checking how another student characterized the same sample. My role is to be available as a reference for keys or to assist with formulating observed differences. Students struggle often with the numbering and advancing statements. I find that checking the sequence within the first few lines is all the correction or direction that most need.

Explain

The key gets written out in a "rough draft" that gets checked by another student. The students can freely adapt the key upon discussion with one another. The interpersonal communication is the stressed skill for this cycle. Students must explain their reasons for separation of the pollen; students must listen to and evaluate each other as well. My role is to monitor the different interactions of pairs. Teachers should stress to students that the draft does not need to result in a uniform key between the two students – it must only function.

Elaborate

The key gets "finished" into a word-processing document. The finished key is proofread and checked to make sure there are no loose ends or dead ends. Another student who has not seen the key yet is assigned to check it using three different pollen samples. The peer review is the last individual communication step before "print" of the key. The teacher can assign a grade based on how well the key is written and how well the peer review is assessed. Although it takes some time in class, this interaction is worthwhile. The students interact in a mutually beneficial critique without the pressure of a grade.

Evaluate

The student draws conclusions from pollen and its relationship to divisions in plants. Upon reviewing one other key from each of the previous phases, each student is asked to reflect on his or her key. Returning to the original questions from *Engagement*: Are the pollens different enough from one another and able to be matched to plant divisions? Can a pollen grain previously unseen be classified and found similar enough to match the existing key?

With several open questions to the class, they realized that some pollen samples are very similar within groups for the keys made throughout class. The images of the pollen from the *Echinacea* project also match this conclusion. Pollen matched along family lines in plants. This leads to the second answer: pollen can be classified and typically will match the plant family pollen in the key. The student keys ranged from simple differences such as color and shape to more specific characteristics, such as numbers of grooves on the long axis and density of surface extensions. When a characteristic was unknown, the students often invented descriptions such as "wall thickness" or "spikyness."

The students responded favorably to the lab exercise. Many were surprised by what the pollens looked like and how their appearance varied between families (e.g., pollen of the thistle *Carduus nutans* is beautiful and purple-spiked, whereas that of the legume *Amorpha canescens* is often colorless and bean-shaped). Both examples appear in Figures 4 and 5. The students used only the online images but requested some slides to reference as they wanted to see more examples of some pollen. I would recommend using slide samples for the "new" or unseen pollen to match to the key. Because of the large relative size of pollen, they are easier than bacteria or microorganisms to find and view with a microscope. It also simulates better the process of amending a key with a "new or novel" specimen.

Both the field activities and the lesson involve inquiry science. I experienced much of the nature of science as a tenuous human endeavor, and I seek to infuse that same interactive human experience within the lessons of my classroom. This small "corner" of the biological world is like all corners – connected to the walls and framework of all that is Biology. High school students enjoy exploring these corners as much as scientists – perhaps enough to become them. The lesson using the pollen images gives students an experience in classifying the "unknown" in nature. In my classroom, I was able to share my experience directly with my students. My goal is to get my students as close as possible to conducting Biology.

○ For Further Inquiry

The pollen library can be found at http://pollen.wikispaces.com, the Echinacea field blog is at http://blog.lib.umn.edu/wage0005/echinacea, and the Echinacea homepage is at http://echinacea.umn.edu.

O Acknowledgments

Thanks for the opportunities and the assigned work to Team Echinacea: Dr. Stuart Wagenius and the researchers (supported by the National Science Foundation (including Grants 0545072, 0544970, 0521245, and 0083468). I also thank Dr. Janet Briggs and the South Dakota Board of Regents Title II Part A grant for the course "Life Science Concepts for K–12 Teachers," and the institution of Great Plains Lutheran High School for encouragement and support.

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