



NC STATE UNIVERSITY



From Natural Selection to Classification: Building on Concepts



Beginner + Grades 9-12

Facilitator's Guide



“The beginning of wisdom is to call things by their proper names.” Confucius

How true, indeed! How could we communicate information, much less build knowledge, without agreement on what to call things?! The world would be chaos! In large part due to necessity, humans have been classifying organisms in one way or another for thousands of years. Not surprisingly, how we classify, and consequently our resulting classification systems, have changed considerably over time. In this series of activities, youth will have the opportunity, to engage in the study of natural selection and the taxonomic process, leading ultimately to the development of their own classification trees, classifications, and taxonomic guides. Rather than employing abstract or artificial creatures to reinforce key concepts, the activities are based on species native to the Southeast, contributing to building local knowledge and a sense of “place” in youth.

The activities in this guide naturally build on one another, but each activity can also be run on its own, depending on facilitator need and educational setting.

The activities begin with an opportunity for youth to emulate natural selection in action. Youth can formulate and test hypotheses of natural selection in acorn herbivory under various habitat scenarios.

From there, youth are virtually transported to North Carolina in 1780, where they will take on the role of a naturalist trying to understand the diversity of oaks. This activity incorporates underlying theory of natural selection and evolution into an applied framework and process for species delimitation. Along the way, youth learn a bit about various associated topics, including oak morphology, wood properties, the organization of a foundational historic taxonomic text (*Species Plantarum*).



Youth will have a chance to learn how to transform morphological data and mathematically model the similarity of species using a distance-based approach.

Youth will also work with native oak species in an introduction to gene sequence alignment for phylogenetic reconstruction through hands-on alignment exercises. Youth will have developed a specific framework for comparing and contrasting different approaches to classification, using tangible examples pertinent to North Carolina.

In the subsequent activity, it is up to youth to develop (and defend!) their own classification.

The objective of the final activity is for youth to learn and properly construct dichotomous identification keys. The scale of the activity can be adapted, depending on time constraints and educational setting, from the very basic of constructing a key to selected plants to that of the development of a local flora (i.e., a practical guide to the plants of a defined region, like school grounds or a small park).

Overall, this curriculum seeks to provide a series of mini-exercises adding dimension to concepts learned in biology class and an opportunity to synthesize basic theory and contemplate issues faced by taxonomists as they seek to provide useful classifications and associated products for the needs of the diverse clientele they serve.

The facilitator's guide, as well as necessary web components and resources, are available at: <http://herbarium.ncsu.edu/4H/>

North Carolina Essential Standards in Science

This High School curriculum addresses the following North Carolina Essential Standards in Biology:

Bio.3.3.1 Interpret how DNA is used for comparison and identification of organisms

Bio.3.4.2 Explain how natural selection influences the changes in species over time

Bio.3.5.1 Explain the historical development and changing nature of classification systems

Bio.3.5.2 Analyze the classification of organisms according to their evolutionary relationships (including: dichotomous keys and phylogenetic trees)

About ten percent of the questions on the 2012–2013 End-of-Course exam in Biology related to the above standards. Material related to these standards is woven throughout the curriculum, but, for emphasis, some important concepts treated in the End-of-Course exam are highlighted in **purple**.

Prerequisites

This curriculum is rated as Beginner+.

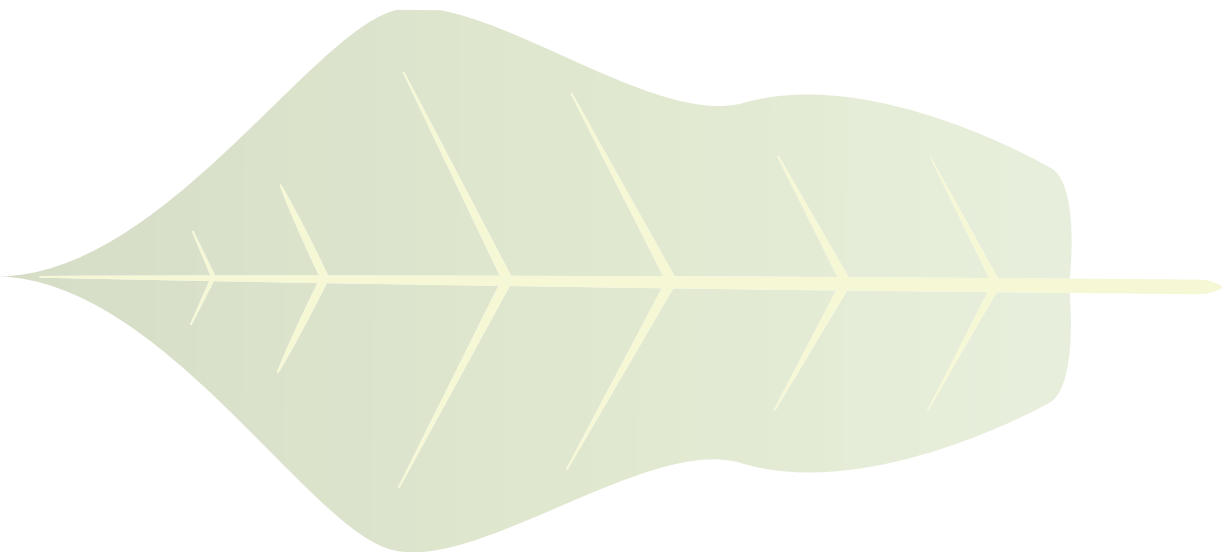
It thus assumes that youth have had some basic introduction, whether through prior readings or class exposure, to the concepts of natural selection, evolution, taxonomy, and classification, and expands upon these concepts through additional learning opportunities.

References or links to online introductory materials are provided, as appropriate, in the activities.

Author

Alexander Krings, Ph.D. serves as Assistant Professor of Plant Biology and Director of the Vascular Plant Herbarium at North Carolina State University.

The central focus of his work is the discovery, taxonomic circumscription, and monography of plants, and the subsequent development of applied resources that facilitate plant identification and related communication. He served on the NC Plant Conservation Scientific Committee for about a decade and is currently serving on the NC Plant Conservation Board. He teaches undergraduate and graduate classes in field botany and nomenclature.



Acknowledgments

These activities were developed as part of the outreach component of National Science Foundation: Collections in Support of Biological Research grant NSF-DBI 1203409 (PI Krings).

Many thanks to **Amy Chilcote** (North Carolina 4-H) for her support through the curriculum development process, the members of the pre-pilot review team (all current or former teachers, or otherwise engaged in curriculum development: **Rachel Clark, Liz Driscoll, Robert Thornhill, and Greg Wilson**), the members of the pilot team (who took the lessons and gave us the feedback needed to make this document accountable to the youth of this state: **Johnston County 4-H, Jones County 4-H, Pitt County 4-H, and Polk County 4-H**), **Autumn Guin** for pilot review evaluation, and **Tabitha Barnes** for illustration and design of the facilitator's guide.

Activity 1 was adapted with permission from DiGiovanni, N. 1989. The chips are down: A natural selection simulation. Evolution & the Nature of Science Institutes. [www.indiana.edu/~ensiweb/].

Many thanks are due to **Andrew Hipp, Marlene Hahn, and Bethany Brown** of the Morton Arboretum for sequencing the ITS region of *Quercus marilandica* specifically for Activity 4 of this curriculum!

Web site banner acorn photographs were taken by **Steve Baskauf** (Vanderbilt University) and **Bruce Kirchoff** (University of North Carolina, Greensboro).

Other images in this work are either in the public domain or used with permission from: **S. Baskauf, W.D. Brush, C.W. Cook, S. Hurst, B. Kirchoff, A. Krings**, the North Carolina State University Herbarium. Please contact the author regarding image use permissions.

Unless otherwise noted, you are free to use as-is, modify, or build upon the lessons herein to suit your needs, provided the end-use is non-commercial and proper attribution is made.

Please cite this resource as:

Krings, A. 2016. From natural selection to classification: Building on concepts. North Carolina State University, Raleigh. 60 pp.

The resource project site can be found at: <http://herbarium.ncsu.edu/4H/>

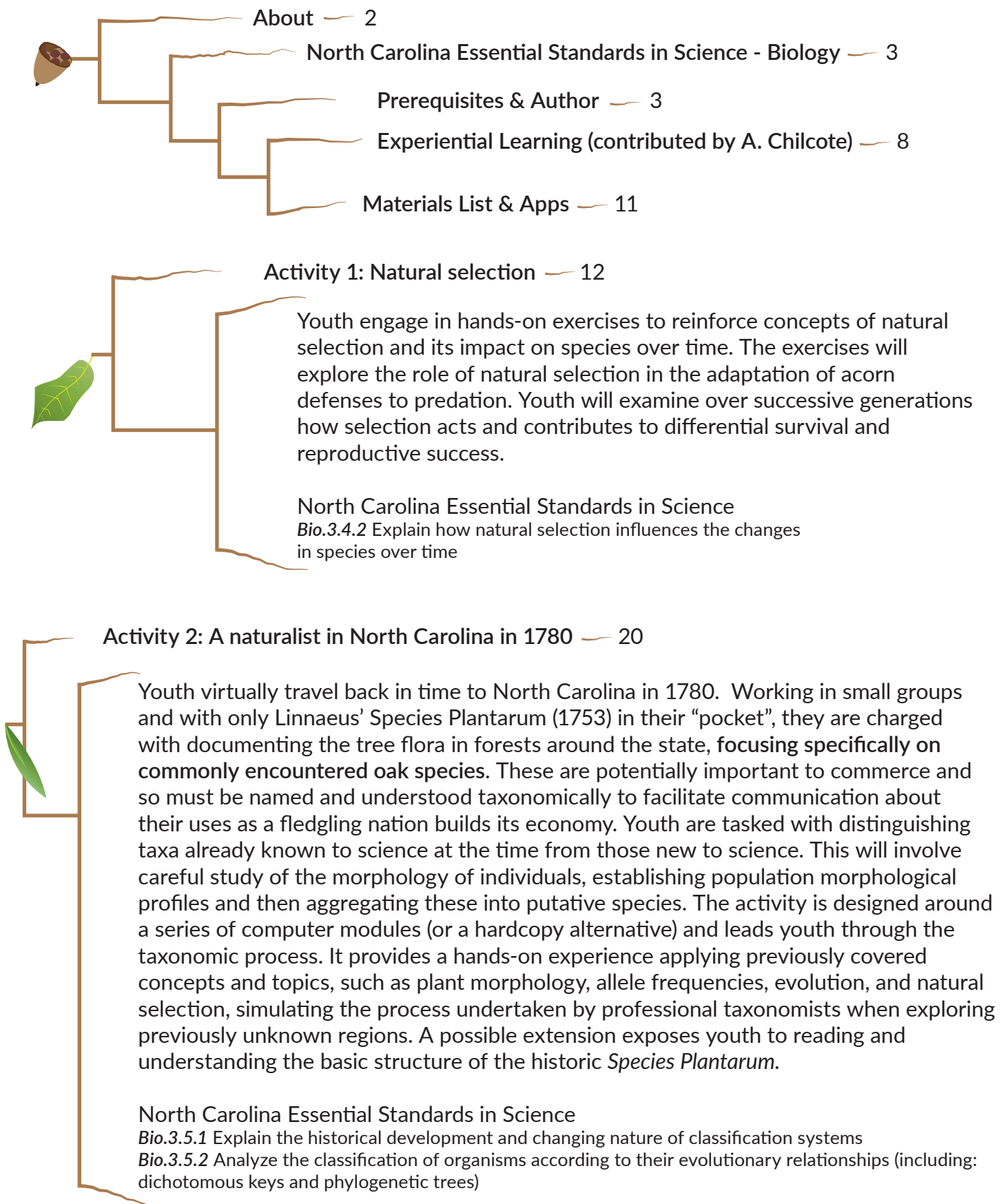
© NC State University & North Carolina 4-H

Funding provided in part by:
The National Science Foundation



Alexander Krings, Ph.D.
Dept. Plant & Microbial Biology
North Carolina State University
Raleigh, NC 27695-7612

Table of Contents



About	2
North Carolina Essential Standards in Science - Biology	3
Prerequisites & Author	3
Experiential Learning (contributed by A. Chilcote)	8
Materials List & Apps	11
Activity 1: Natural selection	12
Youth engage in hands-on exercises to reinforce concepts of natural selection and its impact on species over time. The exercises will explore the role of natural selection in the adaptation of acorn defenses to predation. Youth will examine over successive generations how selection acts and contributes to differential survival and reproductive success.	
North Carolina Essential Standards in Science <i>Bio.3.4.2</i> Explain how natural selection influences the changes in species over time	
Activity 2: A naturalist in North Carolina in 1780	20
Youth virtually travel back in time to North Carolina in 1780. Working in small groups and with only Linnaeus' <i>Species Plantarum</i> (1753) in their "pocket", they are charged with documenting the tree flora in forests around the state, focusing specifically on commonly encountered oak species . These are potentially important to commerce and so must be named and understood taxonomically to facilitate communication about their uses as a fledgling nation builds its economy. Youth are tasked with distinguishing taxa already known to science at the time from those new to science. This will involve careful study of the morphology of individuals, establishing population morphological profiles and then aggregating these into putative species. The activity is designed around a series of computer modules (or a hardcopy alternative) and leads youth through the taxonomic process. It provides a hands-on experience applying previously covered concepts and topics, such as plant morphology, allele frequencies, evolution, and natural selection, simulating the process undertaken by professional taxonomists when exploring previously unknown regions. A possible extension exposes youth to reading and understanding the basic structure of the historic <i>Species Plantarum</i> .	
North Carolina Essential Standards in Science <i>Bio.3.5.1</i> Explain the historical development and changing nature of classification systems <i>Bio.3.5.2</i> Analyze the classification of organisms according to their evolutionary relationships (including: dichotomous keys and phylogenetic trees)	

Table of Contents (cont.)

Activity 3: Building a phenogram — 30



Youth are fast-forwarded in time to the present and have the opportunity, again working in small groups, to build a tree depicting the morphological similarity among the taxa studied in Activity 2. Taxonomic classifications have changed considerably over time, as science has progressed and we have come to better understand taxa and their relationships. Today, taxonomists utilize a number of tools to understand relationships among taxa. In this activity, youth will transform a character matrix applicable to the species in Activity 2 into a distance matrix that will then serve as the basis for developing a classification tree showing morphological relationships. The activity will expose youth to one of the different ways species relationships can be modeled and its applications.

North Carolina Essential Standards in Science

Bio.3.5.2 Analyze the classification of organisms according to their evolutionary relationships (including: dichotomous keys and phylogenetic trees)

Activity 4: A tree is only as strong as its roots: An introduction to gene sequence alignment — 40



Youth will be again working with native oak species in this introduction to gene sequence alignment for phylogenetic reconstruction through hands-on alignment exercises using nucleobase cards. Concepts of alignment scoring are introduced and youth will have the opportunity to score and evaluate their own alignments. Phylogenies are presented based on two common alignment algorithms to prompt discussion on the impact of alignments in reconstruction, as well as the differences in interpretation of phylogenies vs. phenograms.

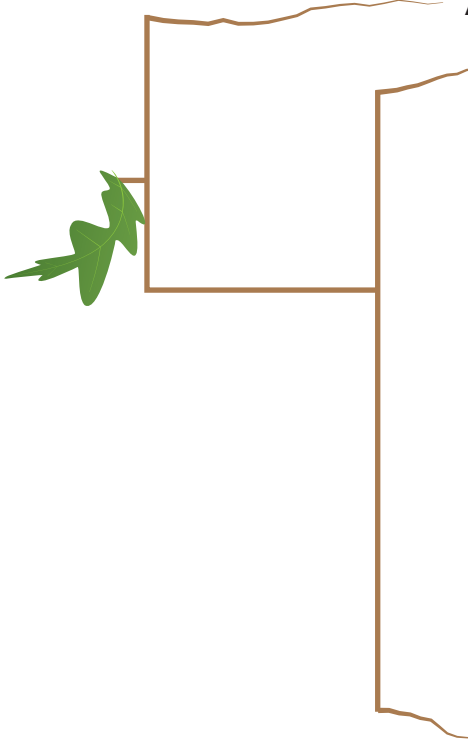
North Carolina Essential Standards in Science

Bio.3.3.1 Interpret how DNA is used for comparison and identification of organisms

Bio.3.5.2 Analyze the classification of organisms according to their evolutionary relationships (including: dichotomous keys and phylogenetic trees)

Table of Contents (cont.)


Activity 5: The changing nature of classification systems — 48



In this activity, youth have the opportunity to take their hands-on experience from the previous activities (identifying previously known species, discovering and naming new species, analyzing their relationships from both a morphological and molecular perspective, etc.) to develop their own classification system for the oaks covered through the activity progression to this point. Youth work in small groups and ultimately must defend their classification (i.e., What is its objective? For example: To be easily memorized? To be predictive? To communicate solely morphological information, morphological relationships, evolutionary relationships, both morphological and evolutionary relationships?). Having worked out various classifications themselves, youth will be better prepared to understand why and how classification systems change.

North Carolina Essential Standards in Science
Bio.3.5.1 Explain the historical development and changing nature of classification systems

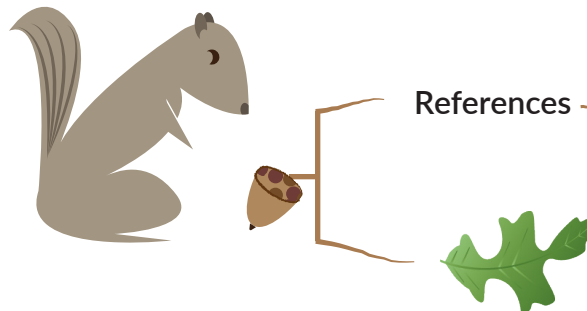
Activity 6: Keys to the flora — 54



The objective of this activity is for youth to develop a guide to the trees of their area. This could be school grounds, backyard, a park, etc.

North Carolina Essential Standards in Science
Bio.3.5.2 Analyze the classification of organisms according to their evolutionary relationships (including: dichotomous keys and phylogenetic trees)

References — 59



Experiential Learning: A Holistic Approach to Learning

Experiential Learning is student centered. It is an active learning process, which first involves youth in an activity, asks them to reflect on the activity to determine what was useful or important to remember, and then tasks youth with understanding how the information they learned applies to their lives now and in the future. This learning process is holistic, allowing youth to consume, apply, and build upon life experiences.

Pfeiffer and Jones' experiential learning model is commonly used in 4-H. However other pioneers such as Kolb provide a wealth of research that emphasizes the importance of the adult facilitator. According to Kolb, the experiential learning model allows the learner to actively participate in what is learned. The facilitator's role is to connect with the student to spark their interest and intrinsic motivation so that the student can gain, revise, or enhance their life skills. The facilitator plays multiple roles during this process, balancing the attention of the learner; engaging with the subject matter; and creating a safe and holistic learning environment.

Tips to think about when creating a welcoming environment for learning:

- Patience is a virtue. When waiting for youth to respond fully, having patience and allowing time for them to think is important.
- You are not the judge. Accept all responses.
- Experiential questions are open-ended to allow for expression. Make sure that you acknowledge youth who respond to you. Not acknowledging all youth can stop the flow of answers and make youth less likely to respond in the future.
- You are not the young person. Don't answer the question.
- Encourage deep conversation. If a young person gives a short response, provide follow up questions to allow for deeper conversation. Examples could be: "Would you provide an example of what you mean?" "Can you tell me more about your thoughts?"
- Avoid yes or no questions. These are not open-ended and stop the flow of conversation.
- Don't lead the youth to answers you want to hear.
- Allow the youth to share their responses in pairs rather than with the entire group.



Experiential Learning: A Holistic Approach to Learning

1. Experience: Let's Do It!!!

Key Reminders to Leader:

- "Sit on Your Hands"
- Observe
- Facilitate to the "Bigger Picture"

Key Objectives for discovery:

- To Explore
- To Examine
- To Construct
- To Arrange

2. and 3. Share and Process

Share: Key Concept Respond

Key Question: "What Happened?"

Processing: Key Concept: Analyze Patterns

Key "What's Important?"

Leader Role

- Allow adequate processing time for sharing
- Use open-ended questions to stimulate thinking and feeling.
- Encourage "pair share" and large group share.

4. and 5. Generalize and Apply

Generalize: Key Concept: Inference

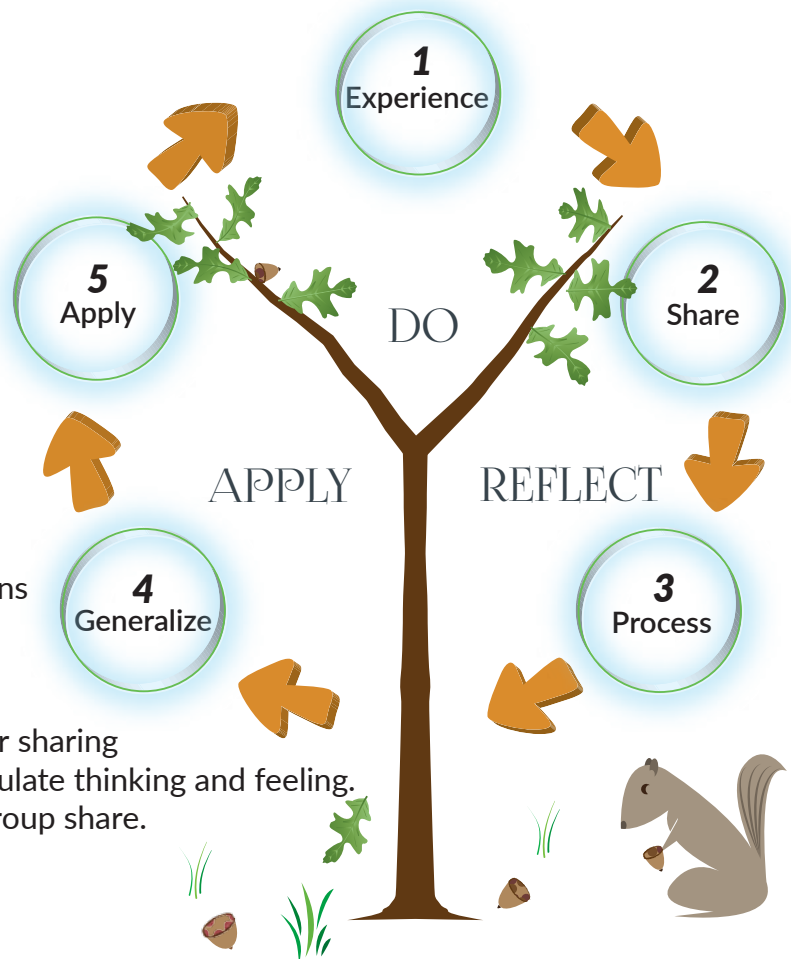
Key Question: "So What?"

Apply: Key Concept: Application

Key "Now What?"

Leader Role

- Facilitate youth finding ways to use what they have learned in new situations.
- Guide youth in making connections between the inner meaning of the activity and the broader world.



Experiential Learning: A Holistic Approach to Learning

Share Questions (What Happened?)

What did you like about this activity?
What part of the activity was the hardest to do?
How did you decide what to choose?
What was the most fun about doing the activity?
How did your group work together?
How did you feel about successfully being able to ____?
What are some decisions you had to make to carry out this activity?
What steps did you go through before you made your decision?
Why do you think everyone in your group didn't agree about?
What was it like to have to make quick decisions?
How did you keep track of everyone's ideas?
Do you think you get more ideas working alone or in a group? Why?
What kind of feelings did you have when group members argued?
What did you observe about the way the groups disagreed (or agreed)?
How did you use your various senses to ____?
What new words did you learn?
Why do you think people have different ideas about what is correct?

Processing Questions (What's Important?)

What did you learn about yourself by doing this activity?
Why is it important to know ____?
How did your group decide ____?
What did you learn as a group that you might not have learned alone?
What were some common themes or thoughts you heard?
How were each person's viewpoints the same?
How were they different?
What problems came up over and over?
What would you do if _____ were to happen?
What was easy or difficult about working with a group to ____?
What did you do if everyone in the group didn't agree on ____?
How did this differ from the way you are usually taught in school?

Give an example of a challenge you had and what you did to solve it.
What works best to get people involved and excited about doing this type of activity?
Why is learning with others sometimes more fun than learning alone?
What suggestions would you have for someone who wanted to ____?

Generalize Questions (So What?)

What other situations like this have you experienced?
Why is it important that each person has his/her own view?
When have you had to ___ before?
Where can you find resources to help you make some of your decisions about __?
When else have you had fun and learned new things at the same time?
Why is it important to have plenty of information before making decisions?
When do you make decisions that require everyone in the group to agree?
What do you do when you don't agree with the group?
What did you learn about your own skill in making decisions?
What did you learn about your own skill in communicating with others?
Describe five ways in which new ideas can be communicated to you?
In what ways do people help each other learn new things?
What are some ways you like to learn?
What did you learn?

Apply. What was Learned?

How would you teach someone about this activity or concept?
What did you learn today that you will be able to use in school....at home?
What did you learn by participating in this process that will help you in the future?
Describe a time when you might need the skills/knowledge you learned today?
What are some other situations when you will need to use the skills you learned today?
What would you do differently if you conducted this activity again?

Materials list



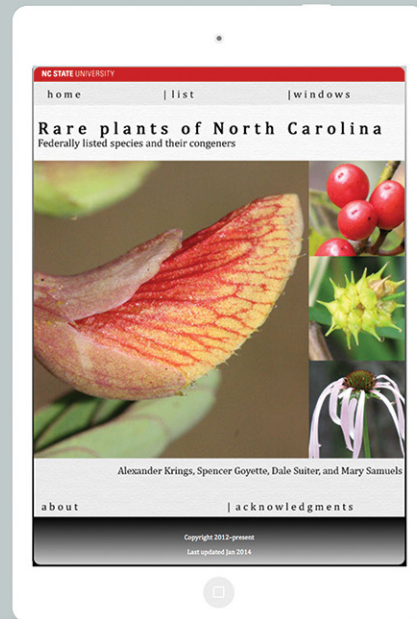
Activity 1	Activity 2	Activity 3
Materials <ul style="list-style-type: none"> ▪ Acorn card decks (1 per youth group; 5 for class of 20) ▪ Habitats (paper or fabric; 5 per class of 20) ▪ Pencils or pens (1 per youth) 	Materials <ul style="list-style-type: none"> ▪ Computers with internet access (1 per youth group) or print-outs of slides from project web site ▪ Pencils or pens (1 per youth) 	Materials <ul style="list-style-type: none"> ▪ Paper (several sheets per youth) ▪ Pencils or pens (1 per youth)
Activity 4	Activity 5	Activity 6
Materials <ul style="list-style-type: none"> ▪ Nucleobase cards ▪ Paper (several sheets per youth) ▪ Pencils or pens (1 per youth) 	Materials <ul style="list-style-type: none"> ▪ Paper (several sheets per youth) ▪ Pencils, pens, or colored markers ▪ Poster board or computers with PowerPoint or other presentation software (1 per youth group) 	Materials <ul style="list-style-type: none"> ▪ Computers or smartphones with internet access and word processing software (1 per youth group; option dependent) ▪ Option 1 only: Digital cameras or smartphones with cameras (1 per youth group; option dependent) ▪ Pencils or pens (1 per youth) ▪ Plant material or species print-outs from project web site (option dependent) ▪ Paper (several sheets per youth)

Learn More:

INTERESTED IN LEARNING MORE ABOUT THE FLORA OF NORTH CAROLINA? TRY THE FOLLOWING WEB APPS:



Samuels, M. and A. Krings. 2012–present. ***Pitcher perfect! Identifying pitcher plants in North Carolina.*** Version 1.0. North Carolina State University, Raleigh. <http://herbarium.ncsu.edu/sarracenia/>



Krings, A., S. Goyette, D. Suter, and M. Samuels. 2012–present. ***Rare plants of North Carolina: Federally listed species and their congeners.*** Version 1.0. North Carolina State University, Raleigh. <http://herbarium.ncsu.edu/rare/>

Activity 1: Natural Selection

Skill Level: Beginner+, ages 14–18

Learner Outcomes:

- (1) Able to apply the scientific method to conduct an experiment
- (2) Able to calculate mortality and recruitment
- (3) Able to graph scientific results and interpret in narrative
- (4) Able to explain the scientific concept of natural selection
- (5) Able to apply the concept of natural selection to understand modern issues (e.g., antibiotic resistance)

Education Standard(s):

North Carolina Essential Standards in Science

Bio.3.4.2 Explain how natural selection influences the changes in species over time

Success Indicators:

Collects and critically analyzes data, works collaboratively in team atmosphere, applies critical thinking to solve problem, explains theoretical concepts

Life Skills: Critical thinking, teamwork, problem solving, learning to learn

Tags: Natural selection, evolution

Time Needed (to complete the activity): 30–90 min (depending on how many habitat variations and rounds are run)

Materials List: Worksheets (template provided), acorn cards (template provided), paper or fabric tablecloth (for "habitat"), writing implements (i.e., pencils), habitats (five; four camouflage pattern: white, green, brown, desert; one floral pattern)

Space (setting needed to conduct the activity): Space for several small working groups

Suggested Group Size: 4 members per group

Introduction:

In 2014, the American Kennel Club recognized 230 breeds of dogs. (AKC 2014)



"Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 die each year as a direct result of these infections." (CDC 2013)

"Three years after the introduction [...], [herbicide-resistant] Palmer amaranth infested 95 to 100% of the area in all fields, resulting in complete crop loss." (Norsworthy et al. 2014)

Although these statements may at first seem different, they are united by a common factor: selection.

The many dog breeds created to date are the direct result of selection and propagation by breeders of certain desirable traits. Emerging resistance to antibiotics is the result of the inadvertent selection for resistant bacteria by over- or misapplication of antibiotics. Emerging herbicide resistance in weeds is the result of over or misapplication of herbicides, resulting in selection of weeds that are genetically resistant.

Simply put: **Selection acts upon genetic variation, which itself is largely the consequence of sexual reproduction and mutation. Individuals with favorable mutations survive and reproduce, thus increasing the number of individuals in a population exhibiting these favorable mutations.** Selection is a powerful directional force and of great consequence to our success as a species. In fact, humans have taken advantage of the directional power of selection for thousands of years, selecting for and breeding desirable traits in everything from dogs, to pigeons, to vegetables, and so on. In fact, without this ingenuity, especially in food crops, we would be unlikely to sustain ourselves at the level that we have. However, as evidenced by statements above, selection can also have dire consequences, and thus the concept remains societally relevant.

Although humans have long been aware of the power of *artificial* selection (the kind involved in all three scenarios noted above), the power of *natural* selection in shaping populations, particularly regarding its effect on evolution of traits and species, was poorly understood until the 19th century. In fact, the articulation of the concept of natural selection and its influence on organisms

Activity 1: Natural Selection

became one of the most transformative concepts to emerge out of the nineteenth century. The concept was laid out in papers by Charles Darwin and Alfred Russel Wallace (Fig. 1.1), jointly communicated to the Linnean Society of London in 1858. However, it was really Darwin's treatise *On the Origin of Species* (1859) that broadly disseminated the idea and contributed to society's changing understanding of biodiversity.

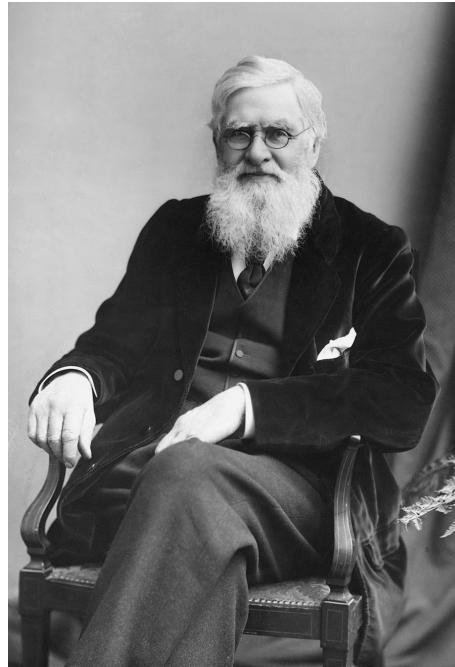
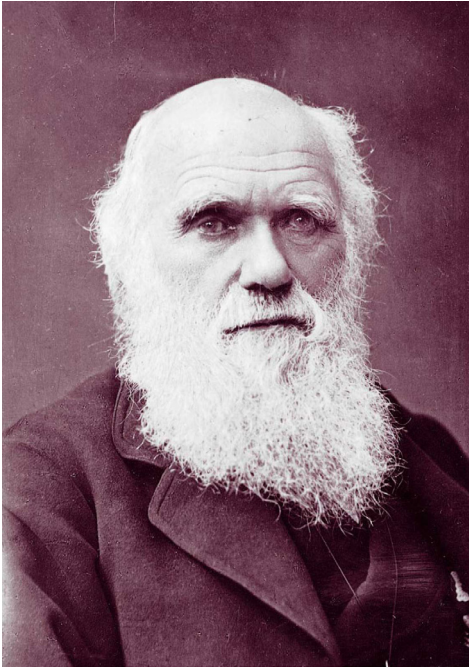


Fig. 1.1 Charles Darwin (left) and Alfred Russel Wallace (right)
Wikimedia Commons/Public Domain

Because evolution is a change of allele frequencies in populations, factors acting upon populations in a selective fashion have the potential power to shape the evolution of those populations. Of course random selection on individuals in a sustainable population has no effect on changing allele frequencies, but selection that consistently targets certain traits, certainly will. Selection does not necessarily result in the evolution of species, (and certainly is not the only evolutionary mechanism), but it has the power to do so. Just as for ecologists interested in understanding the interaction of organisms, for taxonomists interested in classifying life on earth, an understanding of natural selection is a key component in the requisite disciplinary theoretical toolbox.

Opening Questions:

To introduce variation and selection, a facilitator might start with a quick show of hands:

“When it comes to donuts, how many of you prefer Dunkin Donuts?
How many Krispy Kreme?”

“When it comes to soft drinks, how many of you prefer Coke? How many Pepsi?
Any other brands?”

“When you go to the fair, what kinds of foods do you go for?
Let's make a graph.”

What's the point of all these questions?

As can be seen from the responses, given a variety of choices, tastes vary distinctly even among small sample sizes like the classroom.

Of course, humans aren't the only ones on this planet with varying tastes and preferential selection.

In fact, we can use selection based on taste to illustrate the process of natural selection, the topic of this activity.

Natural selection is a fundamental concept that can explain many phenomena we see around us, including why acorns from some trees are more bitter than others, how plants become resistant to herbicides, how insects become resistant to pesticides, and how bacteria become resistant to antibiotics.

Activity 1: Natural Selection

Background information:

Engage in hands-on exercises that reinforce concepts of natural selection and its impact on species over time. Explore the role of natural selection in the evolution of acorn defenses to herbivory. Examine over successive generations how selection acts and contributes to differential survival and reproductive success.

- Before the Activity (steps facilitator must take prior to the activity):

Be familiar with concepts of natural selection and evolution. Be familiar with squirrel biology. Have necessary materials on hand.

- Let's Do It! (steps to conduct the activity): Facilitator guides youths through the activity, answering questions as needed. The objective of the activity is provide youth the opportunity to formulate hypotheses regarding the interaction of organismal characteristics and habitat, carry out an experiment, analyze results, and apply findings to modern issues (e.g., herbicide resistance, antibiotic resistance, etc.).

Facilitator Concepts:

Find brief background information on natural selection, population genetics, and evolution at Khan Academy:

[khan academy, variation in a species](#)

[khan academy, introduction to evolution and natural selection](#)

[khan academy, biology crash course](#)

[khan academy, biology crash course \(2\)](#)

Squirrel Biology:

See the following for interesting reading on squirrel biology!

[Yarrow, G. 2009. Gray squirrel biology and management. Clemson Cooperative Extension Fact Sheet 13.](#)

[Smallwood, P.D. and W.D. Peters. 1986. Grey squirrel food preferences: The effects of tannin and fat concentration. Ecology 67: 168-174.](#)

Term and Concept Discovery

Allele: any of the alternative forms of a gene at a given locus.

Generation: a group of individuals born and living contemporaneously.

Genotype: all or part of the genetic constitution of an individual or group.

Herbivory: the state or condition of feeding on plants.

Mortality: the number of deaths in a given time or place.

Natural selection: a natural process that results in the survival and reproductive success of individuals or groups best adjusted to their environment and that leads to the perpetuation of genetic qualities best suited to that particular environment.

Phenotype: the observable properties of an organism that are produced by the interaction of the genotype and the environment.

Predation: the act of killing and eating other animals.

Recruitment: the act of adding new individuals to a population or subpopulation by reproduction.

Survivorship: the number or proportion of survivors (as of an age group or population)

Variation: divergence in the structural or functional characteristics of an organism from the species or population norm or average.

*Definitions derived from: Merriam-Webster (2014).

PLAY AN ONLINE NATURAL SELECTION GAME DEVELOPED BY THE NATURAL HISTORY MUSEUM OF LONDON:

[The evolution experience](#)

Activity 1: Natural Selection

Activity 1:

- (1) Divide youth into five groups of four, with each group receiving a different fabric or paper habitat (camouflage or floral pattern suggested, as indicated in Materials List). For smaller class sizes or as an alternative, the activity outlined below can also be repeated successively using a different habitat each time. [As an alternative to a printed habitat, youth could be asked to design their own habitats on poster board].
- (2) Lay paper or fabric habitat on table.
- (3) Each group should receive an acorn card deck consisting of an equal number of cards in the following four categories (25 each is suggested): Small/Tasty, Small/Bitter, Large/Tasty, Large/Bitter (i.e., 100 cards total for each group; five decks per class of twenty).
- (4) After youth see the habitats and the card deck, they should be asked to develop a hypothesis regarding expected results of predation. For example, one hypothesis might be that in winter conditions (white camouflage habitat), rates of predation of large and small acorns will be the same, but that in fall conditions (brown camouflage habitat), large tasty acorns will suffer higher predation rates than small tasty acorns, as they visually stand out more.
- (5) Once hypotheses have been developed, youth proceed testing them through the activity that follows:
 - (a) Designate one person to serve as tally keeper and acorn distributor. While the rest of the group (predators [e.g., squirrels]) has their backs turned, that person will distribute the acorn cards over the habitat face down (recommended: distribute five cards in each of the four acorn categories for a total of twenty cards on the table at the start).
 - (b) Predators turn around one at a time and have one second to pick an acorn card off the table. Depending on the hypothesis, predators can either just remove the card or use the back of the card as a guide as to whether to remove or not. For example, if the back indicates "tasty", the acorn is predated and removed from the habitat. If the back indicates "bitter", the acorn is rejected and put back on the habitat. Once a predator makes this determination, they must turn their backs and wait for their next turn. Proceed with card removal until five cards are left on the table.
 - (c) Analyze the remaining acorn cards and tally in the worksheet.
 - (d) Assume that each surviving acorn germinates and the resulting tree produces three viable offspring acorns. For each surviving acorn, the tally keeper must then place three additional acorn cards on the table (being careful to ensure that all offspring match the parental phenotypes).
 - (e) While the predators again turn their backs, the tally keeper redistributes the acorn cards randomly on the habitat.
 - (f) Repeat the predation and reproduction process as many times as desired (at least two rounds are recommended), each time tallying the results on the worksheet.

Talk it over

Share

- What did you like about this activity?
- What was your group's plan of action in choosing the acorns?
- What new words did you learn?

Reflect

- What did you learn as a group that you might not have learned alone?
- What was challenging in this activity?
- What could make this activity better?

Generalize

- Can you provide five examples of selection in our environment?
- Can you elaborate on those examples as to how they have impacted our environment?

Apply

- How would you teach someone about this activity or concept?
- What did you learn that can be applied to other parts of your life?

Activity 1: Natural Selection



Fig. 1.2. Cards of large acorns for use in Activity 1 with five different habitat backgrounds (from top left: brown, desert, floral, green, and white). To generate cards of small acorns for this activity, reduce images by 50%.

See Figs. 1.3 and 1.4 for corresponding text for the back of the cards.

Activity 1: Natural Selection

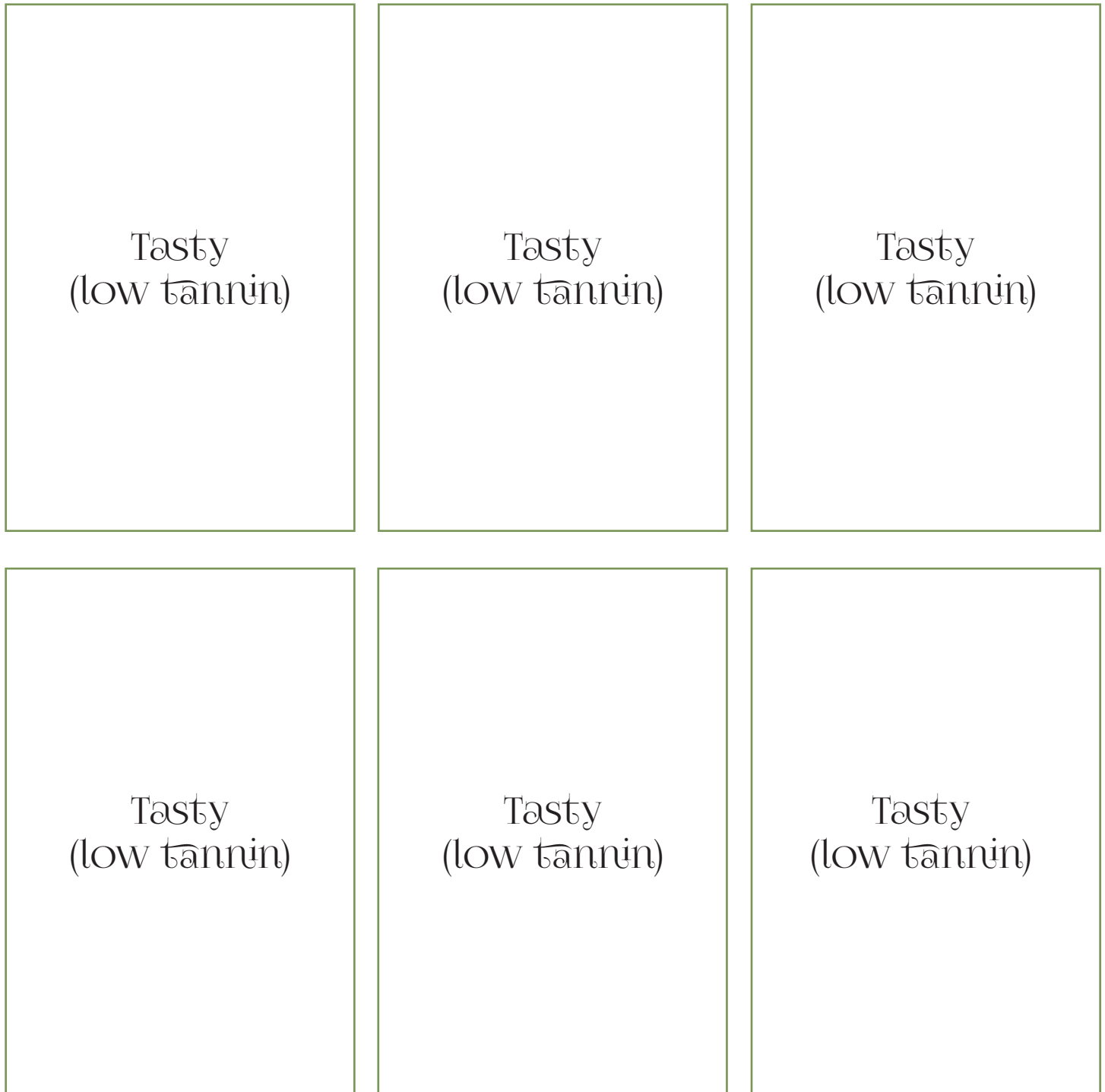


Fig. 1.3. Background text for cards of large, tasty acorns (low tannin content) for use in Activity 1. To generate text for cards of small acorns for this activity, reduce by 50%.

See Fig. 1.4 for corresponding text for the back of cards for bitter acorns (high tannin content).

Activity 1: Natural Selection

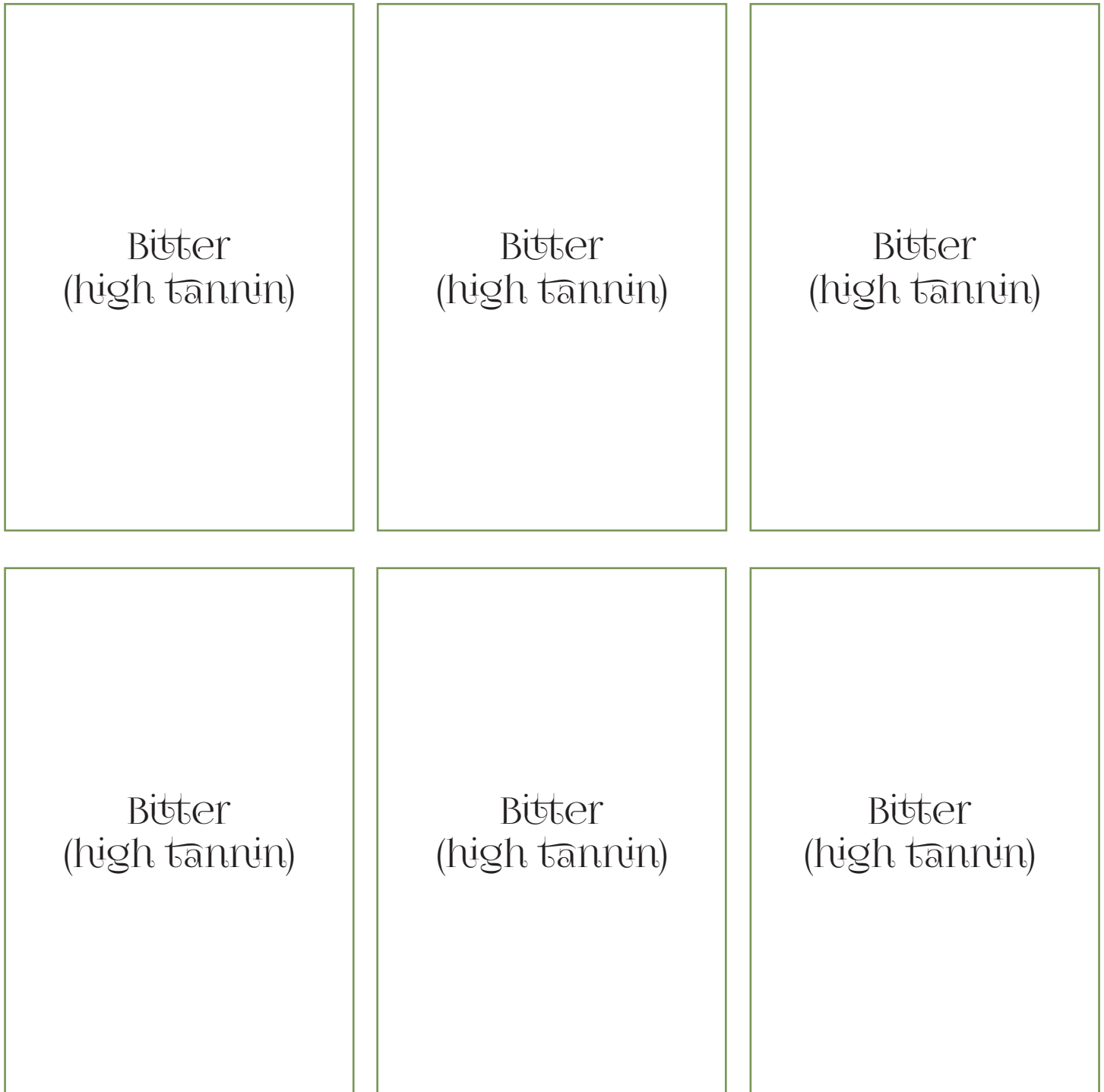


Fig. 1.4. Background text for cards of large, bitter acorns (high tannin content) for use in Activity 1.
To generate text for cards of small acorns for this activity, reduce by 50%.

See Fig. 1.3 for corresponding text for the back of cards for tasty acorns (low tannin content).

Activity 1: Natural Selection

Activity 1: Natural Selection: Student Worksheet

Name:

Date:

	Acorns small/tasty	Acorns small/bitter	Acorns large/tasty	Acorns large/bitter
Number at start				
Number predated in round 1				
Number left after 1st predation				
Total number after 1st reproduction (i.e., left from predation + their offspring*)				
Number predated in round 2				
Number left after 2nd predation				
Total number after 2nd reproduction (i.e., left from predation + their offspring*)				
Number predated in round 3				
Number left after 3rd predation				

*Offspring = number of individuals remaining of a certain type x 3.

Activity 2: A Naturalist in North Carolina in 1780

Skill Level: Beginner+, ages 14–18

Learner Outcomes:

- (1) Describe and analyze morphological variation
- (2) Analyze historical text and apply correct scientific names
- (3) Apply background knowledge of theory of evolution to aggregate populations into putative species

Education Standard(s):

Bio.3.5.1 Explain the historical development and changing nature of classification systems

Bio.3.5.2 Analyze the classification of organisms according to their evolutionary relationships (including: dichotomous keys and phylogenetic trees)

Success Indicators: Able to describe and analyze morphological variation, understands organization of *Species Plantarum*, able to apply background theory to analyze data to reach taxonomic conclusion

Life Skills:

Teamwork, problem solving, decision-making, critical thinking

Tags: Taxonomy, evolution, species, nomenclature

Time Needed (to complete the activity): 50 min (90 min if advanced activities are employed)

Materials List:

Paper, pencils or pens, matrix handout, and computers with access to internet

If access to the internet is limited, the facilitator has the option to download the pictures from the website and use them as hands-on manipulative

Space (setting needed to conduct the activity): Space for several small working groups, each with a computer that is connected to the internet

Suggested Group Size:

4 members per group

Introduction:

Oaks

The oaks (*Quercus* spp., Fagaceae) have and continue to be an important group in both ecologic and economic respects.

Although some oaks are mere shrubs, the vast majority become long-lived trees in forests, providing numerous important ecological services, including, but not limited to carbon binding, air cleaning, wildlife shelter, and mast (fruit) production. Economically, oaks are important for many industries, particularly housing and furniture.

We now recognize a number of different evolutionary lineages in oaks (e.g., Red Oaks, White Oaks, etc.). Species in these lineages often exhibit different biologies and characteristics. Thus, White Oaks tend to mature their fruit (acorns) in a single growing season, whereas Red Oaks take two growing seasons.

Fruit maturation is often correlated with tannic acid or tannin content. High tannin content makes acorns taste bitter. Acorns of Red Oaks, for example, generally have a higher tannin content which makes them less desirable for some animals. Acorns of White Oaks generally have a lower tannin content which makes them more palatable to animals.

Because of this, these acorns can face higher predation.

Facilitator Species Concepts:

Find brief background videos on species and natural selection at Khan Academy:

[Khan Academy- species](#)

[Khan Academy- crash course biology- 113](#)

Fun facts:

SOME INDIGENOUS TRIBES IN NORTH AMERICA WELL EXPLOITED THEIR KNOWLEDGE OF PALATABLE SPECIES AND INCORPORATED SPECIES IN THE WHITE OAK GROUP INTO THEIR DIET (USUALLY STILL WASHING OUT TANNINS IN STREAMS BEFORE PROCESSING).

TANNIN IS FOUND IN TEA. WHAT OTHER FOODS HAVE TANNIN?

Learn More

For more on activities on species and defining species, see:

evolution.berkeley.edu- defining species-

Interested in seeing oaks in the wild? Why not visit a State Park? For more information see:

<http://www.ncparks.gov>

Going outside and laying on the grass underneath a tree(s) can provide a different lens to the taxonomy of trees. Allow youth to lie down and look up and identify what they see.

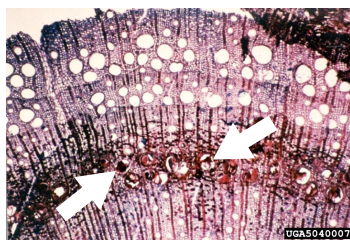


Activity 2: A Naturalist in North Carolina in 1780



The wood properties also differ between Red and White Oaks (as well as the species in each group).

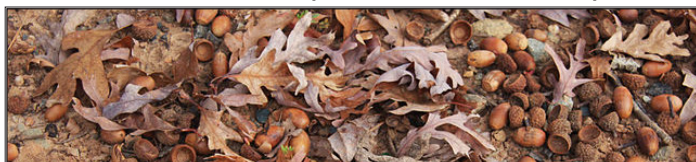
Thus, if you were a competent cooper in the 1700s (i.e., a barrel maker), you would prefer White Oak wood for barrels because of its tighter "cooperage". Cooperage is a term that refers to the leakiness of wood. In oaks, tight cooperage is a factor of vessel elements being plugged with tyloses. Vessel elements are the components that collectively form the vessels that in turn together with tracheids constitute the water conducting tissue of the xylem in hardwoods (vessels are absent in most softwoods like pines, which only exhibit tracheids). Tyloses (sing. tylosis) are bubble-like outgrowths of the vessels that can plug up the vessels themselves (and thus prevent water flow) as the sapwood turns to heartwood. So, if you were making a barrel for wine, you would prefer a wood with tight cooperage, if you were making a container to ship dry goods, you would prefer a wood with slack cooperage.



Tyloses in oak- Joseph OBrien/USDA Forest Service/Bugwood.org licensed under CC BY 3.0

The wood properties of many species vary, so even today, it is of tremendous interest to be able to identify species correctly. Of course, accurate identification is of importance to any endeavor or discipline in need of communicating information about any organism.

The discipline charged with classification and the identification is taxonomy. Today, taxonomy is quite complex, its tools spanning both traditional morphological approaches to complex next-generation gene sequencing. But at the root of it all, remains the basic question: What is a species?

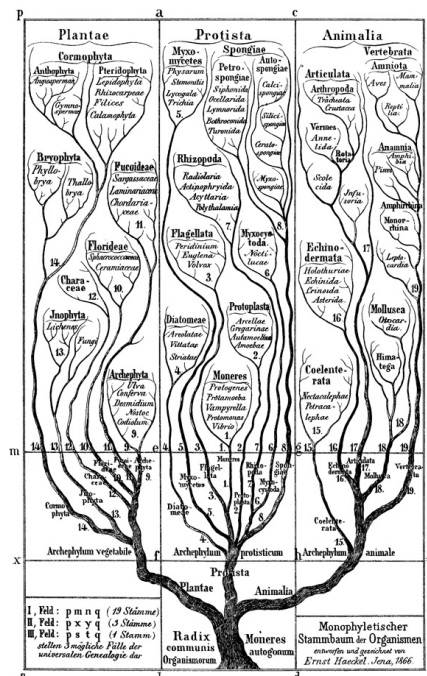


White oak (*Quercus alba*) acorns/ Dcrrsr/ https://commons.wikimedia.org/wiki/File:White_oak_Quercus_alba_prolific_acorns.jpg/ licensed under CC BY 3.0

Species

Many people find it difficult to articulate what a species is, although most would likely feel they would recognize one if they saw it.

This would likely hold as true in the 1700s as today, although our understanding of species has changed considerably, especially following Darwin and the revolution in our understanding of genetics and evolution. However, organisms and their properties (such as modes of reproduction) vary enormously--consider the difference between bacteria, sunflowers, and humans, for instance--making it difficult to establish one all encompassing definition of a species. Nonetheless, many have tried and dozens upon dozens of species concepts exist. While some concepts--such as the Biological Species Concept--appear to work reasonably well with some groups (e.g., animals), they don't apply very well at all for other groups (e.g., plants; botanists, in fact, have largely abandoned the Biological Species Concept). Application of species concepts is often confounded by a failure to distinguish the theoretical (what a species is) from the applied (how a species is recognized).



Tree of Life, according to Haeckel, E. H. P. A. (1866) Haeckel/Wikimedia Commons/Public Domain

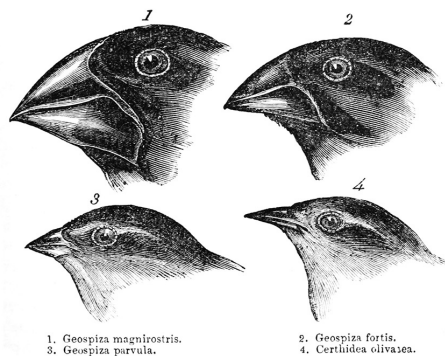
Activity 2: A Naturalist in North Carolina in 1780

Today, most plant taxonomists would likely agree that species are distinct evolutionary lineages (and subspecies, incompletely diverged lineages). One influential species concept has been termed the Phylogenetic Species Concept (Nixon & Wheeler 1990). This concept holds that a species is the smallest aggregate of populations discernable by a unique combination of character states. Most alpha-level plant taxonomists, that is those actively describing new species, rather than working on genus or family level classifications, essentially follow this species concept, whether explicitly or not, in determining and classifying species.

Why the emphasis on populations? Because the basic unit of evolution is the population. Evolution, in genetic terms, is defined as a change in allele frequencies in a population. Thus, an individual cannot evolve. However, populations can evolve as the forces of evolution, such as natural selection, act on individuals, shaping the frequency of alleles in the population over time.

Populations that are isolated, for instance, may diverge in characteristics due to a variety of factors, such as purely stochastic (random) events or adaptation to new ecological niches.

A classic example of selection and diversification can be seen in Darwin's finches. Fixed differences in allele frequencies between populations are evidence of divergence and the lack of homogenizing gene flow. On a gross morphological level, it is thus a valid null hypothesis that species level divergence has



Darwin's Finches.
Charles Darwin/Wikipedia Commons/Public Domain.

happened between populations (or aggregates of populations) when individuals in those populations share the same unique combination of character states (these being the proxies for "underlying" alleles).

Botanical nomenclature

The rules that govern the application of plant names are known as the International Code of Nomenclature (ICN) and can be rather complex.

When scientists describe new species, they must carefully follow the rules laid out in the ICN or their description will not be valid. At the species level, the rules require that a name be a latinized binomial, consisting of a genus and specific epithet. The epithet must agree in gender with the genus name: hence the difference in epithet endings, such as *Quercus rubra* and *Acer rubrum*. Authors of new species names must additionally provide a diagnosis or description of the new species. A diagnosis is a short statement in which the key characteristics distinguishing a new species from morphologically similar or related species are provided. A description, in contrast, does not reference other species, but merely is a highly structured and detailed characterization of the morphology of the species.

In addition, authors of new species names must cite a voucher specimen that shall serve as the standard for the application of the new name and the herbarium (or herbaria, if there are duplicate specimens) in which the specimen is deposited.

Voucher specimens that serve as the standard for names are known as type specimens. Types are invaluable as without them (1) there would be no proof that a species described in a publication actually exists and (2) it would be impossible to conclusively determine whether a newly found organism belongs to a previously described species or a new species.

Activity 2: A Naturalist in North Carolina in 1780

Background information:

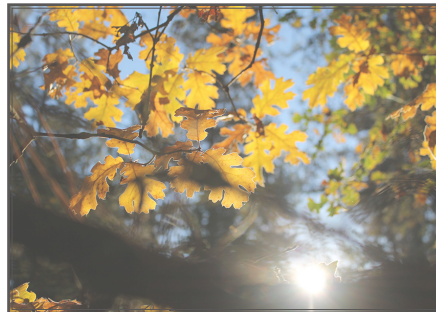
Introduction. In this activity, youth travel back in time to North Carolina in 1780, just a few years after the colonies declared their independence from Britain and a few years prior to the adoption of the Constitution. The United States was a fledgling nation, building its economy. Then, as now, natural resources were of great importance. Wood was used for a variety of products and applications. However, not all wood exhibits the same properties (have you ever wondered why white oak is preferred for making barrels or sugar maple wood for bowling alleys?). Then, as now, research into basic properties of natural resources, such as wood, relies on accurate taxonomic identification, as does the communication of findings to build businesses and the economy.

For this exercise, youth take on the role of taxonomists in 1780, charged with identifying and classifying the trees of North Carolina, so that others may use this information in research and commerce. Naturally, there was no Audubon or Peterson's field guide at this time. The only timely reference is Carl Linnaeus' *Species Plantarum* (1753). Using the web site accompanying this resource (or, as an alternative, laminated slides from the site), youth will virtually visit several forests around North Carolina and study the oaks they find. Based on this, they are charged with answering the following questions:

- (1) How many oak species are there among the trees you've seen in the virtual forest?
- (2) How do you know they are species?
- (3) How do they differ?
- (4) How many oak species were known to western science in 1780 (i.e., worldwide)?
- (5) Were all the oaks you encountered known to science in 1780? If there are any new species, how do you know they are new?

[Advanced: (6) What are the names of the oak species you encountered? What are the names of the species unknown to Linnaeus?

You can identify the species using the dichotomous keys in *Trees of North Carolina* (TNCWG 2014). For more information on dichotomous keys, see Activity 6: Figs. 6.3, 6.4.



Question #6-

Names of North Carolina oaks encountered in the activity.

Species named by Linnaeus in 1753 are indicated by an asterisk; all other species were named after his *Species Plantarum* was published.

*Quercus alba**
Quercus marilandica
Quercus michauxii
*Quercus nigra**
*Quercus prinus** [now *Q. montana*]
Quercus stellata

Opening Questions:

To introduce the activity, a facilitator might ask the class to reflect on English as a language.

English is spoken in numerous countries around the world, but does it all sound the same? Is the vocabulary the same?

What are some examples of how English sounds in Australia, the United Kingdom, Canada? Youth can be additionally prompted to provide examples of regional differences in spoken English or vocabulary in the U.S. itself.

Can youth identify the prominent romance language contribution to English (just think of the many words ending in -ity, -tion, -ment, etc.)? Yet, how is it possible, that with all these differences and influences, English around the world is still considered distinctly English?

How do you recognize English as different from French when heard or written? Origin, syntax, and vocabulary, right?

Now let's turn to the plant world.

How do you recognize an oak from a pumpkin? What are the characters that help distinguish the two? Seems fairly straightforward. But, how do you recognize one oak from another? That can get trickier. Is it important? Sure, because oaks have different properties that are useful to different industries, not to mention their ecological role. In fact, being able to distinguish among species on earth is important in general because of their role in maintaining the biosphere, as well as their direct utility to us.

Although distinguishing species can be rather tricky in some cases, requiring a high degree of specialization and understanding of modern molecular and morphological analysis methods and tools, much progress can be made at the basic morphological level.

In this activity, we'll put ourselves in the shoes of a naturalist in North Carolina in 1780 and see if we can make heads or tails of the diversity in oaks we find as we travel through the state.

The common thread to keep thinking about is "what makes a species a species."



Activity 2: A Naturalist in North Carolina in 1780



Background Information Continued:

The discovery method of species under the Phylogenetic Species Concept has been termed Population Aggregation Analysis (Davis & Nixon 1992). The process is relatively simple and youth will have an opportunity to practice it in this lesson.

The first step is to create a matrix of individuals and their characteristics for each site (*Table 2.1*). Characteristics need to be chosen carefully to be meaningful in the analysis. If all individuals share a character, then it has no discerning power.

Once the character matrix is completed for each individual for each site, it is important to group recurring combinations of characters into profiles for each site (*Table 2.2*).

Finally, recurring profiles are analyzed for non-varying (fixed) characters (these evidence of fixation of underlying alleles). Only unique combinations of fixed characters are useful in distinguishing species under the Phylogenetic Species Concepts.

Thus, in our example, two species are present (*Table 2.3*): Species A (recognized by the fixed characters of tight bark and 2 yr fruit maturation) and Species B (recognized by the fixed characters of platy bark and 1 yr fruit maturation). The depth of the leaf lobe sinuses is not fixed across aggregate profiles in our example and thus cannot be used as a discerning character.

Term and Concept Discovery* –

Allele: any of the alternative forms of a gene that may occur at a given locus.

Classification: systematic arrangement in groups or categories according to established criteria.

Cooper: a person who makes or repairs wooden casks or barrels.

Microevolution: the change in allele frequency in a population.

Evolutionary lineage: descent in a line from a common progenitor.

Individual: existing as a distinct entity.

Morphology: the form and structure of an organism or any of its parts.

Nomenclature: a system of names for things.

Phloem: a complex tissue in the vascular system of higher plants that consists mainly of sieve tubes and elongated parenchyma cells usually with fibers and that functions in translocation and in support and storage.

Population: the total of individuals occupying an area or making up a whole.

Species: a group of organisms that are similar and can produce.

Tannin: a reddish acid that comes from plants, is used in making ink and leather, and occurs in various foods and drinks (such as wine).

Taxonomy: orderly classification of organisms.

Tracheids: a long tubular pitted cell that is peculiar to xylem, functions in conduction and support, and has tapering closed ends and thickened lignified walls.

Tyloses (sing. tylosis): bubble-like outgrowths of the vessels that can plug up the vessels themselves (and thus prevent water flow).

Type: in botany, a plant name standard (usually a specimen, but sometimes an illustration)

Vessel elements: components that collectively form the vessels, that, in turn, together with tracheids, constitute the water conducting tissue of the xylem in hardwoods.

Vessels: a conducting tube in the xylem of a vascular plant formed by the fusion and loss of end walls of a series of cells (vessels are absent in most softwoods like pines, which only exhibit tracheids).

Xylem: a complex tissue in the vascular system of higher plants that consists of vessels, tracheids, or both usually together with wood fibers and parenchyma cells, functions chiefly in conduction of water and dissolved minerals but also in support and food storage, and typically constitutes the woody element (as of a plant stem).

*Definitions primarily derived from: Merriam-Webster (2014).



Activity 2: A Naturalist in North Carolina in 1780



Background Information Continued:

- Before the Activity (steps facilitator must take prior to the activity): Be familiar with the theory of evolution, speciation, oak morphology, population aggregation analysis, and Carl Linnaeus. Review the provided excerpt from *Species Plantarum* and the associated text.

Arrange youth in small groups. Provide computer access for each group. Navigate to the site:

<http://herbarium.ncsu.edu/4H/activity2.htm>

- Let's Do It! (steps to conduct the activity): Facilitator guides youth through the activity, answering questions as needed.

Activity 2:

- (1) Receive context for the activity (1) from the facilitator (e.g., let's travel back to 1780!), who should briefly discuss *Species Plantarum* (background text provided on curriculum web site), botanical nomenclature, and typification. See inset for advanced classes.
- (2) Visit at least three virtual forests (or more, at the discretion of the facilitator), sort the images of characters from individual trees at those sites into the boxes, with each box representing a species.
- (3) Develop a matrix of morphological and habitat characters for each population encountered (this will help make decisions about whether one population found at one site is the same species as another population found at a different site). See **Table 2.1** for an example. It should be possible to determine at least nine characters (including habitat) from the images provided. Look particularly for variation in the bark, leaves, acorns, and occurrence in the landscape. A handout on oak morphology is provided to help students apply domain specific terminology (**Fig. 2.3**). For a fully scored matrix of characters by species, see **Table 1** in the Instructor's Resource for **Activity 3**.
- (4) Think about why a species is a species, in the context of what is known about evolution, alleles, and morphology. Then, determine how many species are found in the virtual forest. At least three of the species were known to Linnaeus in 1753. Which ones are they? What species were unknown to Linnaeus in 1753? Use the dichotomous keys on the web site *Trees of North Carolina* (<http://herbarium.ncsu.edu/tnc/>) to identify all species encountered. See inset for answers.

Advanced Classes:

For advanced classes, facilitators can briefly provide the appropriate context for the activity and then set youth loose to explore *Species Plantarum* on their own for a specified amount of time. Facilitators should guide youth to examine in particular how the work is organized.

The introduction already notes the importance of stamens and carpels in organization, but, to get comfortable navigating through the work, youth can be put to tasks such as figuring out how many different "anther" sections Linnaeus used and how to read an individual species account (i.e., where is the genus name, where is the specific epithet, how many oak species were known to western science in 1780, etc.; see Fig. 2.1 for an explanation).

Each group can be given a copy of the labeled excerpt handout (Fig. 2.2) and be asked to correctly label each part (or at least described what each component is). As most students are probably not familiar with Latin, it may be useful to provide them with this link to a botanical latin dictionary, such as: <http://alumnus.caltech.edu/~hollin/botany/latin/dictionary.html>.

Talk it over

Share

How many species did you find?
Could you identify them all using *Species Plantarum*? Why or why not?

Reflect

What problems came up over and over? How did you (or your group) work through them. How do you know a species is a species? What evidence is used to scientifically justify the recognition of species?

Generalize

What did you learn about your own skill in making classifying decisions?
Observation is a key component to classifying. What did you learn by observation?

Apply

What would you do differently if you conducted this activity again? Explain why?



Activity 2: A Naturalist in North Carolina in 1780



Table 2.1 Sample plant character matrix for two sites and three characters.

	Bark	Leaf lobe sinuses	Fruit maturation
Site 1			
Plant A	tight	shallow	2 years
Plant B	tight	shallow	2 years
Plant C	platy	shallow	1 year
Plant D	platy	shallow	1 year
Site 2			
Plant E	platy	shallow	1 year
Plant F	tight	shallow	2 years
Plant G	tight	shallow	2 years
Plant H	platy	deep	1 year

Table 2.2 Population profiles based on data from Table 2.1.

	Bark	Leaf lobe sinuses	Fruit maturation
Site 1			
Profile 1.1 (based on plants A & B)	tight	shallow	2 years
Profile 1.2 (based on plants C & D)	platy	shallow	1 year
Site 2			
Profile 2.1 (based on plants E & H)	platy	shallow OR deep	1 year
Profile 2.2 (based on plants F & G)	tight	shallow	2 years

Table 2.3 Aggregated population profile based on data from table 2.2.

	Bark	Leaf lobe sinuses	Fruit maturation
Species A			
Profile 1.1 (based on plants A & B)	tight	shallow	2 years
Profile 2.2 (based on plants F & G)	tight	shallow	2 years
Aggregate profile:	tight	shallow	2 years
Species B			
Profile 1.2 (based on plants E & H)	platy	shallow OR deep	1 year
Profile 2.1 (based on plants C & D)	platy	shallow OR deep	1 year
Aggregate profile	platy	shallow OR deep <i>[no fixed characters]</i>	1 year

Activity 2: A Naturalist in North Carolina in 1780

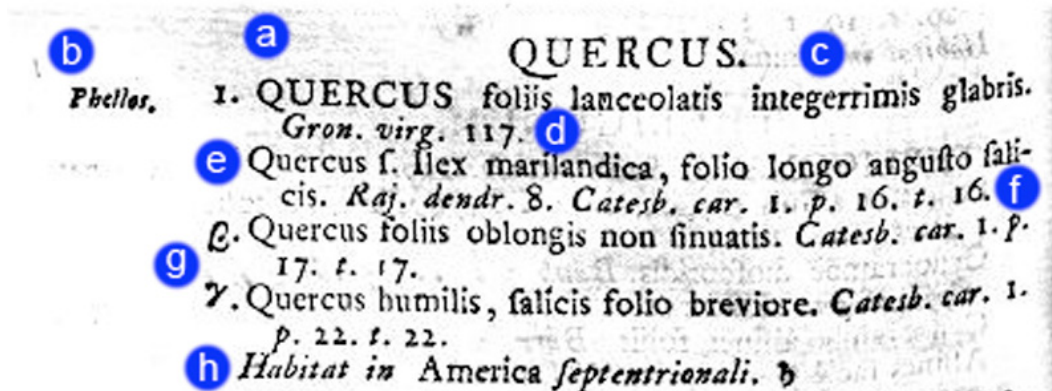


Fig. 2.1. Explanation of sample oak species account in *Species Plantarum* (Linnaeus 1753).

Note that the genus name for the oaks (i.e., *Quercus*) was the same at the time of Linnaeus as it is now. When Linnaeus wrote his species accounts, he followed a repetitive format. Once understood, even without knowing all the Latin terms, the format is relatively easily navigated.

Linnaeus numbered each species under a genus separately. Thus in the excerpt shown here, we are seeing the first species in his treatment of oaks.

Following the species number, one will always find the name of the genus in all caps (in this case, *QUERCUS*; see **a**). The specific epithet (which is the second part of the scientific binomial) was always written in the margins (see **b**). This was the hallmark of Linnaeus' work and one of the reasons his work was chosen as the starting point of plant nomenclature: Unlike those before him, who either inconsistently provided epithets or only provided descriptive names (see **c**), Linnaeus consistently provided an epithet in the margins (**b**), in addition to the descriptive names popular during his time (see **c**).

Thus, today, we would recognize the species treated here as *Quercus phellos* (genus = *Quercus*; specific epithet = *phellos*). Sometimes the specific epithet is referred to as the species name, but this is not technically correct. The correct species name is the genus and specific epithet combined (i.e., *Quercus phellos*).

Descriptive names (**c**) are just short phrases of distinguishing characters. These types of names worked just fine when few species were known, but as more and more species were discovered to western science (particularly with on-going explorations of the New World and Asia), it quickly became intractable to continue updating these names to reflect increased knowledge. The descriptive name for *Quercus phellos*, as shown above, is *Quercus foliis lanceolatis integerrimis glabris* (**c**), which means Oak with leaves (*foliis*) lanceolate (*lanceolatis*) entire (*integerrimis*) and without hairs (*glabris*). The abbreviated text following the descriptive name (i.e., *Gron. virg.* 117; **d**) is a bibliographic citation to another work where this name has appeared.

Synonyms, like **e** for example, are always listed immediately below the descriptive name and before the habitat (**h**). In our example, there are three synonyms, each with their own bibliographic citations (e.g., **f**).

Linnaeus frequently used symbols as shorthand to denote varieties or subspecies (**g**).

Habitat and/or distribution (**h**) was provided for all species and is often a useful clue when trying to determine for instance how many species were known from a particular continent at the time of his writing. In our case, *Quercus phellos*, is shown as being known from *America septentrionali* or northern America.

Activity 2: A Naturalist in North Carolina in 1780

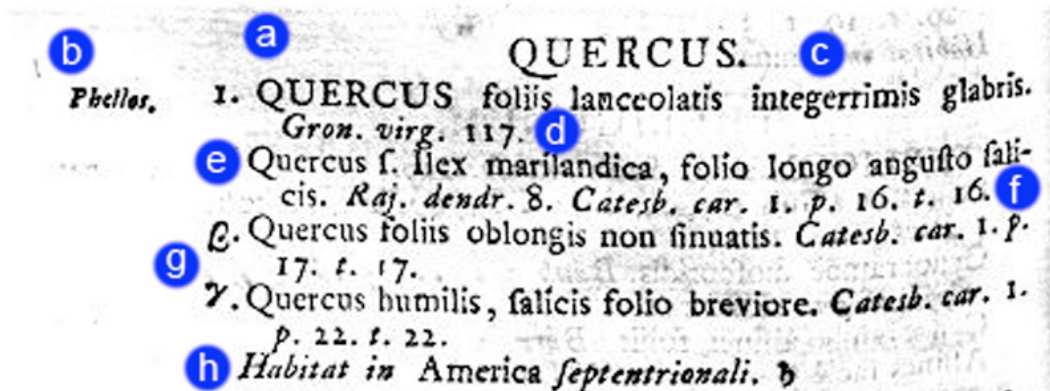


Fig 2.2 Youth handout:

Sample oak species account in *Species Plantarum* (Linnaeus 1753). Please provide an explanation of each labeled feature:

a: (example): *Quercus* is the genus name.

b: What part of the scientific name does *Phellos* represent? _____

c: The phrase *QUERCUS foliis lanceolatis integerrimis glabris* (oak with leaves lanceolate, entire, and smooth) represents what kind of botanical name? _____

d: What is *Gron. virg. 117*? Is it part of the scientific name or a bibliographic citation (circle one).

e: This phrase looks similar to the one above. Is another species listed here?
Is it different from the one above? _____

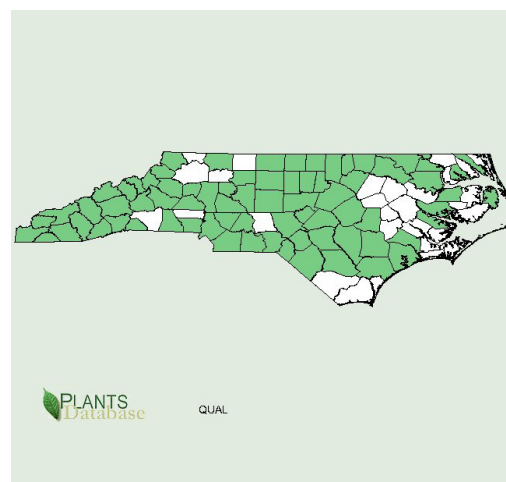
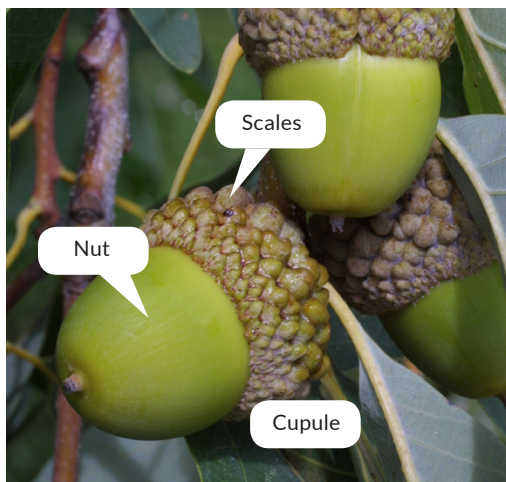
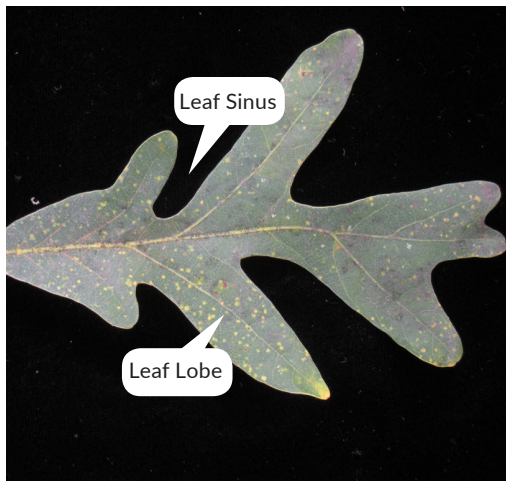
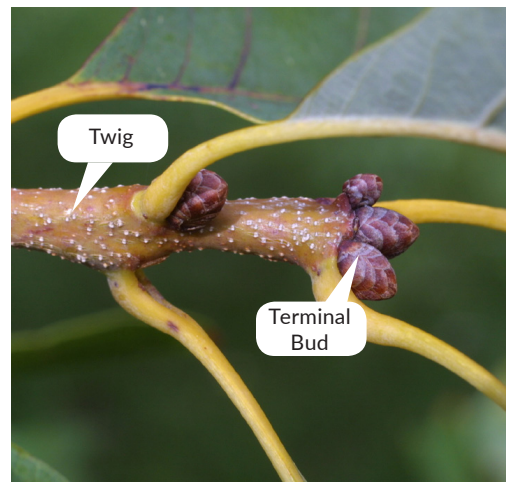
f: What is *Raj. dendr. 8 Catesb. Car. 1. p. 16, t. 16*? Is it part of the scientific name or a bibliographic citation (circle one).

g: What do you think the greek characters represent?

h: What is the informational purpose of this phrase? _____



Activity 2: A Naturalist in North Carolina in 1780



Quercus alba

(Common; mesic to xeric forests; Mt, Pd, CP; Fl: Apr, Fr: Sep-Nov [of the same year])

Fig. 2.3. Youth handout: Some morphological features of oaks, using white oak as an example (*Quercus alba*). From: Trees of North Carolina Working Group (TNCWG). 2014–present. *Trees of North Carolina. Version 1.0.* North Carolina State University, Raleigh.

<http://herbarium.ncsu.edu/tnc/>



Activity 3: Building a Phenogram

Skill Level: Beginner+, ages 14–18

Learner Outcomes:

- (1) Transform attribute data into a distance matrix
- (2) Calculate species distances and join branches to build a tree of relationships

Education Standard(s):

North Carolina Essential Standards in Science
Bio.3.5.2 Analyze the classification of organisms according to their evolutionary relationships (including: dichotomous keys and phylogenetic trees)

Success Indicators: Critically analyzes and transforms data, calculates dissimilarity distances of single and composite operational taxonomic units, mathematically builds a phenetic tree

Life Skills: Critical thinking, teamwork, problem solving

Tags: Phylogenetic and phenetic trees, classification, taxonomy

Time Needed (to complete the activity): 45 min

Materials List:

Worksheet (provided), paper, writing implements (pencils or pens)

Space (setting needed to conduct the activity): Space for several small working groups

Suggested Group Size: 2 members per group

Introduction:

One can mathematically model similarity in relationships among entities in just about any category—screws, bread clips, pens, cell phones, bees, slime molds, and so on. Modeling relationships, whether explicitly mathematically or intuitively, and subsequently naming entities based upon similarity can be a powerful organizing principle. Imagine the difficulty of visiting the hardware store looking for a replacement slotted woodscrew and having to hunt through a random assortment of different fasteners, rather than being able to browse a selection organized by type! Or visiting a library to check out the latest NY Times Best Seller, only to find books arranged completely randomly on the shelves! How would you ever find what you are looking for? This problem is particularly acute when the entities involved in a particular “collection”, whether hardware store inventory of fasteners or library collection of books, potentially number in the hundreds, thousands, or more.

Taxonomists have been occupied with the problem of modeling relationships and developing a nomenclature reflective of classification that most efficiently allows communication and retrieval of information since the dawn of the discipline. This issue has become particularly important in modern times, as the number of species known to science has increased dramatically. Some recent estimates put the number of plant species alone between ca. 215,000 and 300,000 (Table 3.1). Early on in the discipline (18th–19th century), classifications were mostly built based upon intuitive or authoritative approaches, but these have been largely replaced by computational approaches (and with much success!).

Table 3.1 Catalogued and predicted number of species on earth.

Species	Earth			Ocean		
	Catalogued	Predicted	±SE	Catalogued	Predicted	±SE
Eukaryotes						
Animalia	953,434	7,770,000	958,000	171,082	2,150,000	145,000
Chromista	13,033	27,500	30,500	4,859	7,400	9,640
Fungi	43,271	611,000	297,000	1,097	5,320	11,100
Plantae	215,644	298,000	8,200	8,600	16,600	9,130
Protozoa	8,118	36,400	6,690	8,118	36,400	6,690
Total	1,233,500	8,740,000	1,300,000	193,756	2,210,000	182,000
Prokaryotes						
Archaea	502	455	160	1	1	0
Bacteria	10,358	9,680	3,470	652	1,320	436
Total	10,860	10,100	3,630	653	1,320	436
Grand Total	1,244,360	8,750,000	1,300,000	194,409	2,210,000	182,000

Predictions for prokaryotes represent a lower bound because they do not consider undescribed higher taxa. For protozoa, the ocean database was substantially more complete than the database for the entire Earth so we only used the former to estimate the total number of species in this taxon. All predictions were rounded to three significant digits.
 doi:10.1371/journal.pbio.1001127.t002

How Many Species Are There on Earth and in the Ocean? by Mora et al. 2011 licensed under [CC-BY-2.5](https://creativecommons.org/licenses/by/2.5/)

Activity 3: Building a Phenogram

When trying to model relationships, one approach, depending on the objective, can be to focus strictly on similarity as an organizing principle. This approach is sometimes referred to as numerical taxonomy or phenetics. The result of a phenetic study is often the creation of a phenogram, a tree-like diagram depicting relationships among taxa based strictly on how similar they are to one another (Fig. 3.1).

Classifying organisms based on similarity has the same advantage evident in organizing fasteners in a hardware store by type. Over time, a number of numerical techniques have been developed. The objective of this activity is not to provide an overview of all techniques, but rather an opportunity to learn and apply one of the most basic techniques: UPGMA (Unweighted Pair Group Method with Arithmetic mean). <https://en.wikipedia.org/wiki/UPGMA>

Phenetics:

While A.J. Cain and G.A Harrison introduced the term "phenetic" in 1960, it was the microbiologist Peter Sneath and biostatistician Robert Sokol who became particularly ardent proponents of phenetic methodology. Their influential text *Principles of Numerical Taxonomy* was published in 1963.

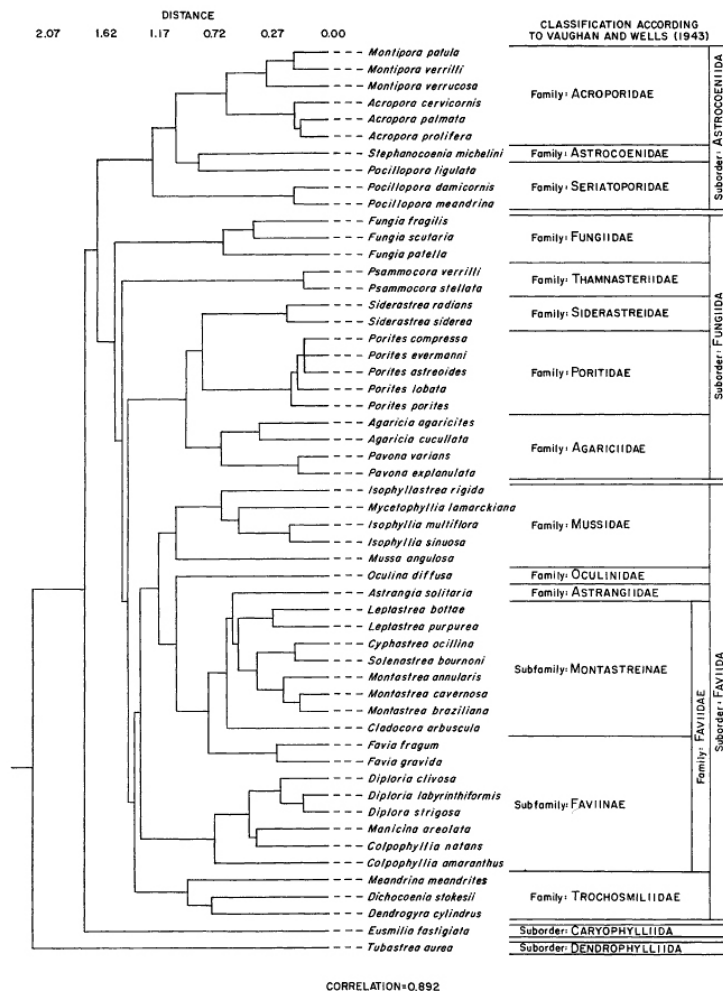


Fig 2.—Distance Phenogram. Cophenetic correlation is below the phenogram. Classification of OTU's by scheme of Vaughan and Wells (1943) is right of phenogram.

Fig. 3.1 Phenogram of Caribbean and Hawaiian reef corals (With permission of the publisher from: Powers, D.A. & F.J. Rohlf. 1972. A numerical taxonomic study of Caribbean and Hawaiian Reef Corals. Systematic Zoology 21: 57; Society of Systematic Biologists).

Suggested Opening to the Lesson:

To lead into this activity, facilitators might set up a series of stations around the classroom and ask youth to sort the items they find by relationship.

For example, items might include types of screws (e.g., flat head, hex head, round head, etc.) and types of nails (e.g., finishing nail, box nail, etc.).

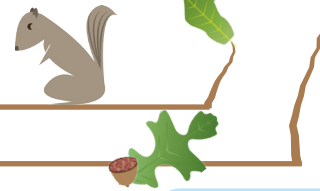
Youth can make a visual sort (classification) by placing the items on a blank sheet of paper and drawing circles around respectively more inclusive categories.

An example of classification by relationship for this set would be two higher-level categories (i.e., two large circles, one for all screws and one for all nails), with each having subcategories [subcircles], perhaps by head shape, length, etc. (of course, other classifications are possible).

The purpose of having youth sort these items is to get them to begin thinking about defining categories and relationships among categories.

Once youth have completed the sorts, facilitators can explain that any type of relationship among categories can be modeled mathematically in a number of ways and that today one of those methods would be introduced.

Activity 3: Building a Phenogram



Background information:

In this hands-on exercise, youth are fast-forwarded in time to the present and have the opportunity, again working in small groups, to build a tree depicting the morphological relationships among the taxa studied in Activity 2. Taxonomic classifications have changed considerably over time, as science has progressed and we have come to better understand taxa and their relationships. Today, taxonomists utilize a number of tools to understand relationships among taxa.

In this activity, youth will transform a character matrix developed in Activity 2 into a distance matrix that will then serve as the basis for developing a tree showing relationships. The activity will expose youth to one of the different ways species relationships can be modeled and its applications, as well as limitations. As youth likely developed a number of different matrices for Activity 2 (depending on the number and kind of characters they scored), it is recommended that facilitators use the matrix provided in the Instructor Resource for Activity 3 to teach how to build a phenogram. This will be more time efficient and ensure that everyone remains on the same page.

Phenogram:

A phenogram based on morphological character states depicts overall morphological similarity among the OTUs, not genetic relationship.

Thus, a question asking which species are most closely related based on the phenogram is a trick question!

The activity need not be run in its entirety (i.e., through all steps), depending on time constraints and educational setting. It is recommended, if possible, that the activity is run at least through Step 5, but running through Step 3 will provide youth experience in all basic aspects of the exercise (i.e., developing a distance matrix, joining the most similar species, and re-calculating the new distance matrix). From there, the steps are repetitive until the entire phenogram is constructed (i.e., iteratively joining species and re-calculating the distance matrix).

- Before the Activity (steps facilitator must take prior to the activity): Be familiar with development of a distance matrix, as well as phenetic tree building.
- Let's Do It! (steps to conduct the activity): Work through the accompanying Instructor resource for Activity 3: Building a phenogram (starts on page 30).

Talk it over

Share

- Which part of the activity was hardest to do?
Which part was the easiest?
- How did your group decide to work together?
What steps did you go through before you made your decision?
- How did you keep track of everyone's ideas?

Reflect

- What did you learn as a group that you might not have learned alone?
- What were some common themes or thoughts you heard?
- What was the most challenging or difficult part of this activity?
Give an example of a challenge you had and how you solve it?
Why did the distance matrix need to be recalculated after each time a species pair or group was joined?
- In the phenogram, **which species are shown to be most closely related genetically?**

Generalize

- How can a phenetic tree (phenogram or dendrogram) be used in classification? What does it emphasize?
- What did you learn by observation?

Apply

- How would you teach someone about this activity or concept?
- How will your new skills help you at home?
At school?
- How could the things you learned today be used to help you in other situations?



Term and Concept Discovery* –

Attributes: an inherent characteristic (e.g., alternate leaf arrangement in oaks, opposite leaf arrangement in maples).

Cladogram: a branching diagrammatic tree used in cladistic classification to illustrate phylogenetic relationships.

Classification: systematic arrangement in groups or categories according to established criteria.

Composite operational taxonomic unit: the theoretical unit formed from the joining of two or more operational taxonomic units in the process of calculating and building a phenetic tree.

Dissimilarity matrix: also known as a distance matrix; a matrix consisting of values that each represent the number of differences in attributes between respective operational taxonomic units.

Distance matrix: also known as a dissimilarity matrix; a matrix consisting of values that each represent the number of differences in attributes between respective operational taxonomic units.

Nomenclature: a system of names for things especially in science.

Operational taxonomic unit: the most basic unit used in a taxonomic analysis, such as individuals or species.

Phenetic: of or relating to taxonomic analysis that emphasizes the overall similarities of characteristics among biological taxa without regard to phylogenetic relationships.

Phenogram: a branching diagrammatic tree used in phenetic classification to illustrate the degree of similarity among taxa.

Phylogenetic: based on natural evolutionary relationships.

Taxonomy: orderly classification of organisms.

*Definitions derived primarily from: Merriam-Webster (2014).

Learn More:

FOR AN ONLINE TUTORIAL IN UPGMA,
SEE EDWARDS & PARKER (2013):

[http://www.southampton.ac.uk/~relu06/
teaching/upgma/](http://www.southampton.ac.uk/~relu06/teaching/upgma/)

Instructor resource for Activity 3: Building a phenogram

How to build a phenogram using UPGMA

A phenogram is the representation of the relationship between entities based on their similarity to one another.

Note that there are multiple methods of calculating phenograms and the body of literature analyzing their appropriateness and effectiveness for different situations is deep. In addition, it is common to refer to the entities in an analysis (species in our case) as **Operational Taxonomic Units (OTUs)**. This terminology will be followed here as well.

In the steps that follow, you will learn how to calculate and develop a phenogram using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). UPGMA is one of the simpler methods to calculate and is thus used here for demonstration purposes.



Activity 3: Building a Phenogram

Instructor resource for Activity 3: Building a phenogram continued

Step 1: Complete a morphological matrix. For the purpose of Activity 3, the use of the completed matrix below is recommended (**Table 1**). Facilitators can provide the matrix to students as a handout or project it using an overhead transparency.

Table 1. Sample morphological matrix for six species of oaks (*Quercus*) of North America

OTUs	Bark (platy vs. ridged vs. polygonal)	Leaf shape (oval/ elliptic vs. cross-like vs. spatulate)	Leaf lobe sinuses (shallow vs. deep)	Lobes (apically disposed vs. throughout)	Leaf tips (lacking bristles or with bristles)	Acorn cup texture (flat vs. knobby)	Cup scale (not fused vs. fused)	Acorn maturation (1yr vs. 2yrs)	Habitat (wet vs. dry)
A (alba)	Platy	Oval/ Elliptic	Deep	Throughout	Lacking	Knobby	Not fused	1	Dry
B (michauxii)	Platy	Oval/ Elliptic	Shallow	Throughout	Lacking	Knobby	Not fused	1	Wet
C (montana)	Ridged	Oval/ Elliptic	Shallow	Throughout	Lacking	Flat	Fused	1	Dry
D (stellata)	Platy	Cross-like	Deep	Throughout	Lacking	Knobby	Not fused	1	Dry
E (nigra)	Tight	Spatulate	Shallow	Apically disp.	With	Flat	Not fused	2	Wet
F (mari-landica)	Polygonal	Spatulate	Shallow	Apically disp.	With	Flat	Not fused	2	Dry

Step 2: Transform the morphological matrix into a distance matrix. A distance matrix is simply a matrix showing the step-wise distance each of your OTUs is from the others. The two axes are labeled the same and represent your OTUs.

You can also think of this as a dissimilarity matrix. For example, for the nine-character matrix above, A and B share all but two characters (i.e., lobe sinuses and habitat are different). Thus, the distance matrix entry for A/B is two (see highlight in **Table 2** below). Naturally, the distance between any OTU and itself is zero, so the entries for A/A, B/B, C/C, etc. will all be zero.

Depending on the class dynamics, facilitators can complete the remaining matrix, working through each species pair, together as a class or in small groups. It is worthwhile to go over a summary of the completed matrix as a class, prior to proceeding with Step 3.

Activity 3: Building a Phenogram



Instructor resource for Activity 3: Building a phenogram continued

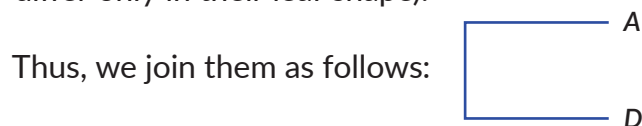
Table 2. Sample distance matrix for six species of oaks (*Quercus*) of North America, based on distances calculated from the morphological matrix in Table 1 above.

	A (alba)	B (michauxii)	C (montana)	D (stellata)	E (nigra)	F (marilandica)
A (alba)	-					
B (michauxii)	2	-				
C (montana)	4	4	-			
D (stellata)	1	3	5	-		
E (nigra)	8	6	7	8	-	
F (marilandica)	7	7	6	7	2	-

Note that only half the matrix is completed in **Table 2**. This was done to save space, as A/B is the same as B/A, C/B the same as B/C, etc. The resulting matrix is called a lower-triangular matrix. Should the entire matrix be completed, you would have a square matrix. Different software packages may differentially require input of lower-triangular or square matrices.

Step 3: Begin joining OTUs and re-calculate the matrix. In order to develop a phenogram, you will need to iteratively join the nearest OTUs and then re-calculate the resulting distance matrix until all OTUs are joined.

The nearest OTUs in our example (or the least dissimilar) are A and D. The distance between these two is just one (they differ only in their leaf shape).



Once OTUs are joined, they are referred to as composite OTUs. Because A and D are now joined, they no longer exist separately in our matrix. Thus, we re-calculate the pertinent distances in the matrix. The process is relatively straightforward.

Let's begin with populating the single cell A,D/B. To achieve this, you will need to obtain the distance AB and DB from **Table 2** and calculate as follows:

$$\begin{aligned}
 \text{distance (A,D),B} &= (\text{distance AB} + \text{distance DB}) \div 2 \\
 &= (2 + 3) \div 2 \\
 &= 2.5
 \end{aligned}$$



Activity 3: Building a Phenogram



Instructor resource for Activity 3: Building a phenogram continued

Table 3. Partially re-calculated distance matrix following joining of OTUs A and D. Note that for demonstration purposes only the cell reflecting the re-calculated distance between the composite OTU A,D to the OTU B has been filled in.

	A,D	B	C	E	F
A,D	-				
B	2.5	-			
C			-		
E				-	
F					-

To complete the rest of the matrix, you will need to calculate all distances to the composite OTU A,D in a similar manner. Thus,

$$\begin{aligned}
 \text{distance (A,D), C} &= (\text{distance AC} + \text{distance DC}) \div 2 = (4 + 5) \div 2 = 4.5 \\
 \text{distance (A,D), E} &= (\text{distance AE} + \text{distance DE}) \div 2 = (8 + 8) \div 2 = 8 \\
 \text{distance (A,D), F} &= (\text{distance AF} + \text{distance DF}) \div 2 = (7 + 7) \div 2 = 7
 \end{aligned}$$



The distance between unjoined OTUs has not changed, so their values are the same as in **Table 2**. The resulting, re-calculated matrix should look like this:

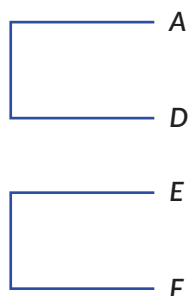
Table 4. Re-calculated distance matrix following joining of OTUs A and D. Bolded values were re-calculated following joining of OTUs A and D.

	A,D	B	C	E	F
A,D	-				
B	2.5	-			
C	4.5	4	-		
E	8	6	7	-	
F	7	7	6	2	-

Depending on the time available and class dynamics, facilitators may wish to continue working through the entire phenogram building exercise as a class or allow individual groups to continue on their own.

Step 4: Join the next pair of OTUs and re-calculate the matrix. From here on, you will repeat the same process as described in **Step 3**, joining in each cycle the nearest OTUs. For our example, the nearest OTUs in the re-calculated matrix (**Table 4**) are E and F (with a distance of two steps from one another).

Thus, we have:



Activity 3: Building a Phenogram



Instructor resource for Activity 3: Building a phenogram continued

Then, we re-calculate the matrix, obtaining distances from the previous matrix (**Table 4**):

$$\begin{aligned} \text{distance (E,F), (A,D)} &= (\text{distance E(A,D)} + \text{distance F(A,D)}) \div 2 = (8 + 7) \div 2 = 7.5 \\ \text{distance (E,F), B} &= (\text{distance EB} + \text{distance FB}) \div 2 = (6 + 7) \div 2 = 6.5 \\ \text{distance (E,F), C} &= (\text{distance EC} + \text{distance FC}) \div 2 = (7 + 6) \div 2 = 6.5 \end{aligned}$$



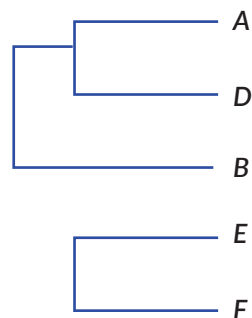
...and get the resulting matrix (**Table 5**):

Table 5. Re-calculated distance matrix following joining of OTUs E and F. Bolded values were re-calculated following joining of OTUs E and F.

	A,D	B	C	E,F
A,D	-			
B	2.5	-		
C	4.5	4	-	
E,F	7.5	6.5	6.5	-

Step 5: Join the next pair of OTUs and re-calculate the matrix. The nearest OTUs in the re-calculated matrix (**Table 5**) are B and the composite A,D (with a distance of 2.5 steps from one another).

Thus, we have:



Then, we re-calculate the matrix, obtaining distances from the previous matrix (**Table 5**):

$$\begin{aligned} \text{distance (A,D,B),C} &= (\text{distance (A,D)C} + \text{distance BC}) \div 2 = (4.5 + 4) \div 2 = 4.75 \\ \text{distance (A,D,B), (E,F)} &= (\text{distance (A,D)(E,F)} + \text{distance B(E,F)}) \div 2 = (7.5 + 6.5) \div 2 = 7 \end{aligned}$$

...and get the resulting matrix (**Table 6**):

Table 6. Re-calculated distance matrix following joining of OTUs A,D and B. Bolded values were re-calculated following joining of OTUs A,D and B.

	A,D,B	C	E,F
A,D,B	-		
C	4.75	-	
E,F	7	6.5	-

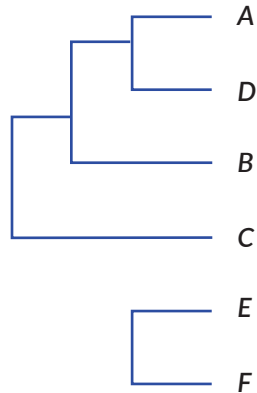
Activity 3: Building a Phenogram



Instructor resource for Activity 3: Building a phenogram continued

Step 6: Join the next pair of OTUs and re-calculate the matrix. The nearest OTUs in the re-calculated matrix (*Table 6*) are C and the composite A,D,B (with a distance of 4.75 steps from one another).

Thus, we have:



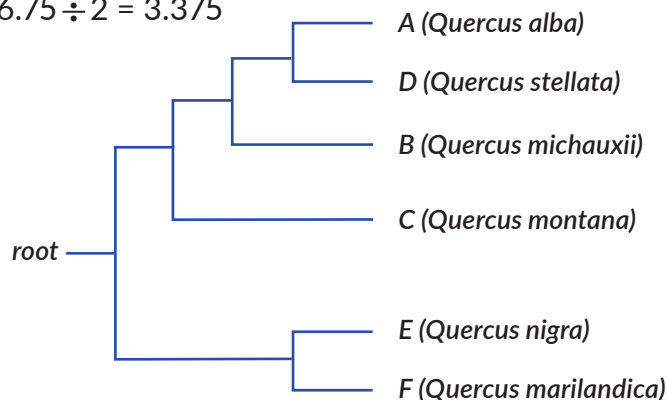
Then, we re-calculate the matrix, obtaining distances from the previous matrix (*Table 6*):
 $\text{distance (A,D,B,C),(E,F)} = (\text{distance (A,D,B)(E,F)} + \text{distance C(E,F)}) \div 2 = (7 + 6.5) \div 2 = 6.75$
 ...and get the resulting matrix (*Table 7*):



Table 7. Re-calculated distance matrix following joining of OTUs A,D,B and C. Bolded values were re-calculated following joining of OTUs A,D,B and C

	A,D,B,C	E,F
A,D,B,C	-	
E,F	6.75	-

Step 7: Join the next pair of OTUs and mid-point root. The only remaining OTUs in the re-calculated matrix (*Table 7*) are the two composites A,D,B,C and E,F (with a distance of 6.75 steps from one another). We can calculate the mid-point root by taking the
 $\text{distance (A,D,B,C)(E,F)} \div 2 = 6.75 \div 2 = 3.375$



Once completed, facilitators may wish to project *Fig. 3.2*, which shows photos of each oak next to the completed phenogram, thus allowing youth to visually connect the abstract of their calculations to the morphology of the trees. If time permits, facilitators can map (or ask youth to map) characters of interest from *Table 1* onto the phenogram, such as which species mature acorns in a single year, which species occur in dry vs. wet habits, etc.

Activity 3: Building a Phenogram

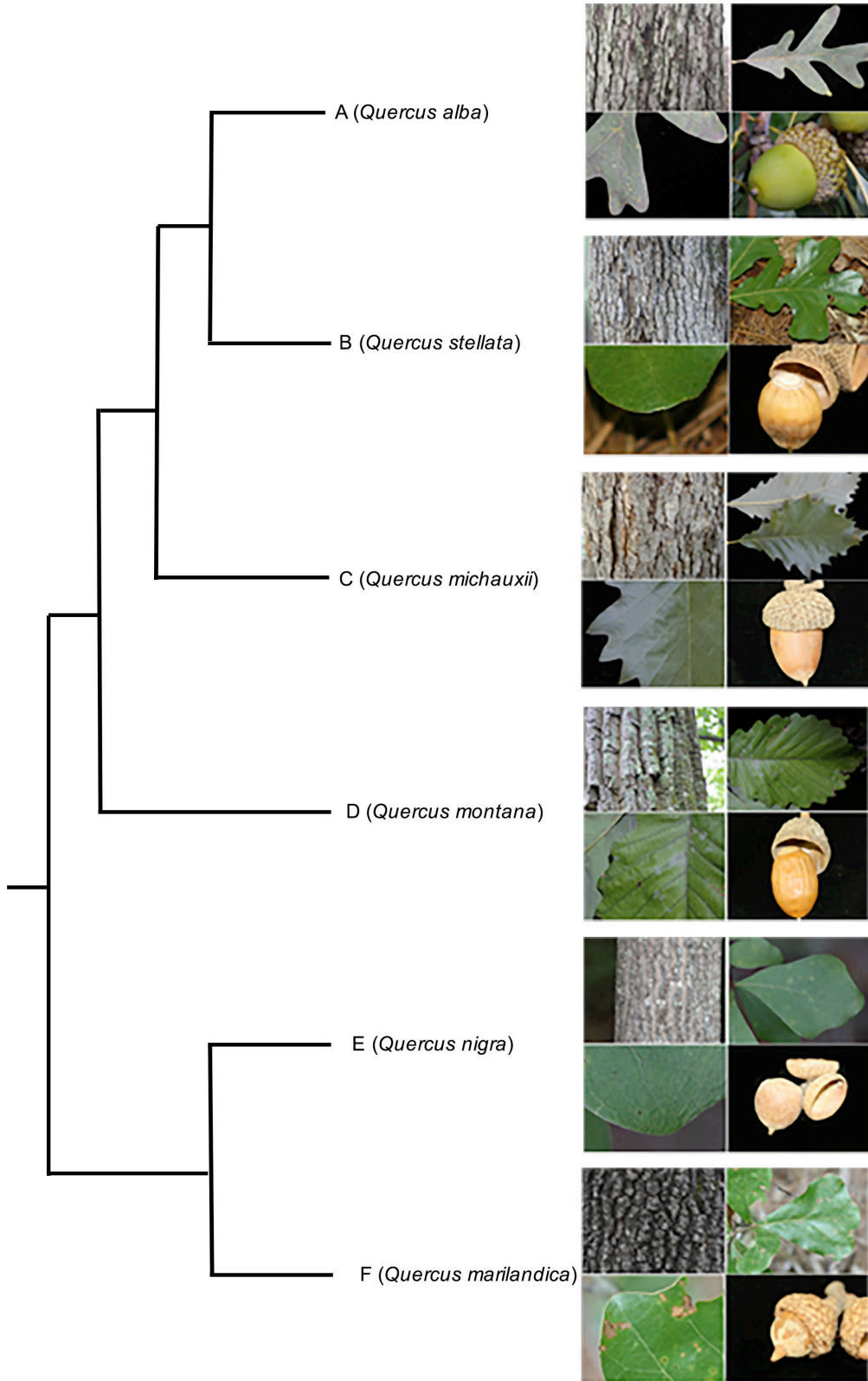


Figure 3.2. UPGMA phenogram of six North American oak species.

Activity 4: A tree is only as strong as its roots: An introduction to gene sequence alignment



Skill Level: Beginner+, ages 14–18

Learner Outcomes:

- (1) Align gene sequences manually
- (2) Score manual sequence alignments using three different scoring approaches
- (3) Evaluate phylogenies resulting from two commonly employed alignment algorithms

Education Standard(s):

North Carolina Essential Standards in Science

Bio.3.3.1 Interpret how DNA is used for comparison and identification of organisms

Bio.3.5.2 Analyze the classification of organisms according to their evolutionary relationships (including: dichotomous keys and phylogenetic trees)

Success Indicators: Aligns gene sequences using indels, as needed; calculates overall scores of alignments under three scoring approaches, using different gap penalty strategies; able to discuss impact of gene sequence alignment on phylogenetic reconstruction

Life Skills: Critical thinking, collaboration, problem solving

Tags: Gene sequence alignment, gap penalty, phylogenetic trees

Time Needed (to complete the activity): 45 to 90 min. (depending on number of sequences aligned)

Materials List: Paper, writing implements (pencils or pens), nucleobase card set

Space (setting needed to conduct the activity): Space for several small working groups; projector (overhead or digital)

Suggested Group Size: 2 members per group

Introduction:

Phylogenies (or evolutionary trees) are today perhaps most widely used to improve taxonomic classifications and investigate character evolution. However, their use is not strictly limited to these areas. In fact, phylogenies are being used in a variety of studies as diverse as reconstructing evolutionary histories of gene families to analyzing whether certain evolutionary lineages are more prone to contain rare taxa, and if so, what are the responsible traits and forces acting on those traits.

Phylogenies differ fundamentally from phenograms in that they represent hypotheses of evolutionary relationships, rather than models of similarity. The process of phylogenetic tree building is often termed phylogenetic reconstruction. Because the emphasis in phylogenetic reconstruction is modeling potential evolutionary relationships, taxa that might be morphologically similar but in fact distantly related would not appear as sister to one another, as they might in a phenetic analysis focused strictly on numerical character distance (*Fig. 4.1*).



Fig. 4.1. Two classification schemes: Phenetic (left; based on morphological similarity) and phylogenetic (right; based on evolutionary relationships). Diagram after Ridley (2003).

A number of computational techniques have been developed over the past few decades to facilitate phylogenetic reconstruction. These techniques include parsimony analysis, as well as probabilistic approaches, such as maximum likelihood and Bayesian analysis. Researchers often combine two or more analysis types when studying a particular problem. Phylogenetic analysis, no matter what technique is employed, is a complicated affair. While the resulting trees can provide important insight into the relationship of taxa, it is important to critically understand how they were built before lending them credence.

The objective of this activity is to focus on the very root of phylogeny building: gene sequence alignment.

Activity 4: A tree is only as strong as its roots: An introduction to gene sequence alignment



Sequence alignment is a crucial step in phylogenetic reconstruction. Correctly aligning nucleobases (hereafter simply bases) is akin to correctly scoring a morphological character matrix.

What if in Activity 3, we had compared the acorn shape of one species to the leaf shape of another? Clearly, the resulting phenogram would not have represented the overall similarity of the two species, because we incorrectly scored the matrix. By not scoring homologous characters, we failed to compare apples to apples and oranges to oranges.

The same principle applies to gene sequence alignment. It is important that homologous gene regions be compared to one another. Unfortunately, sequence alignment can be more complicated than morphological character scoring. Gene sequence variations can result from a number of factors, including, but not limited to point mutations, unequal crossover during meiosis (leading to strings of nucleotides being inserted or deleted), and DNA slippage during replication (resulting in nucleotide string repetition).

Today, increasingly powerful algorithms, some based on models of gene evolution and even secondary structure, are employed by scientists to guide sequence alignment. As an introduction to this area and basis for future learning, it is useful to begin with some hands-on exercises in manual gene sequence alignment.

Background information:

In this hands-on exercise, youth have the opportunity to (1) manually align gene sequences using a card set of bases and indels, and (2) score their alignments, based on three different scoring approaches (Constant, Linear, and Affine). Rather than using artificially contrived gene sequences, youth will align selected sections of the sequence from the nuclear ribosomal internally transcribed spacer (ITS) region of two or more oaks covered in the preceding activities.

- Before the Activity (steps facilitator must take prior to the activity): Understand how phylogenetic reconstruction differs from phenogram building, be familiar with sequence alignment, including the three scoring approaches employed below (constant, linear, and affine; for more information see https://en.wikipedia.org/wiki/Gap_penalty).
- Let's Do It! (steps to conduct the activity): Facilitator guides youths through the activity, answering questions as needed.

Nucleobases:

Nucleobases (*bases*) are nitrogen containing compounds and one of the three basic components of nucleotides, the other two being a five carbon sugar and at least one phosphate group. **Nucleotides in turn are the basic subunits of DNA.** DNA nucleotides are classified in two groups: purines (adenine and guanine) and pyrimidines (cytosine and thymine). **In a double-strand of DNA, adenine binds to the complementarily shaped thymine, and guanine binds to cytosine. In RNA, thymine is replaced by uracil.**

Algorithms:

The accuracy of alignment algorithms is tested using analyses of simulations with known computer generated sequences.

See, for instance: Nuin et al. 2006. The accuracy of several multiple sequence alignment programs for proteins. *BMC Bioinformatics* 7: 471.

Opening Questions:

Turning genes to trees: How do you reconstruct evolutionary relationships based on gene sequence data?

Scientists utilize a variety of methods, each with its own advantages and limitations...but where do you start?

Activity 4: A tree is only as strong as its roots: An introduction to gene sequence alignment

Activity 4:

(1) Youth should work in groups and organize the individual base cards (Fig. 4.2) into the order provided below. Facilitators should gauge the level of their class and the time available. At a minimum, it is recommended that the sequence from the two bolded species be aligned. For advanced classes, it is recommended that three or more sequences be aligned. A sample alignment of the following species using ClustalW can be seen in Fig. 4.4. (note: this figure is a reference only for the facilitator and should not be shown to the youth ahead of their own attempts at aligning the sequences below).

Quercus alba	CCCTGCCCCCTCTGCGCAAACCTCTTTTACCCTGCGACCT
<i>Quercus marilandica</i>	CCCTGCCCCCTCTGCGCAAACCTCTTTTACCCTGCGACCT
<i>Quercus montana</i>	CCTGCCCCCTCTGCGCAAACCTCTTTTACCCTGCGACCT
Quercus nigra	CCCTGCCCCCTCTGCGCAAACCTCTTTTACCCTGCGACCT
<i>Quercus stellata</i>	CCCTGCCCCCTCTGCGCAAACCTCTTTTACCCTGCGACCT

(2) Youth should align the sequences to maximize the number of matches in bases, using the "hyphen" cards as needed to make their alignments. Hyphens are used in alignment software programs and represent indels (insertion/deletion events in the genome).

For example:

Species	Unaligned sequence	Aligned sequence
Species 1	A A G T A C	A A G T A C - -
Species 2	A G T C A A	A - G T - C A A

(3) Once all groups have finished their alignments, the facilitator can ask youth to identify and report to the class (can be written on board) the number of transitions or transversions in their sequences. If this topic has not yet been covered in class, this would be an ideal time to do so. The facilitator should also discuss indels (what are they, how do they occur) and the importance of minimizing gaps in alignments (see insets). Subsequently, scoring should be introduced as a means of recognizing the optimality of one alignment over another.

There are a number of different scoring approaches. For the purposes of this activity, three rather simple approaches will be covered, but youth should be made aware that mathematical progress is advancing rapidly in this area and that considerably more sophisticated computational models are now employed in alignment software. The three gap penalty scoring approaches covered here are: (a) *Constant*, (b) *Linear*, and (c) *Affine* (a very widely used approach).

Card alternative:

As an alternative to the nucleobase cards, pipecleaners and mini clothespins, available at most craft stores, can be used (Fig. 4.3).

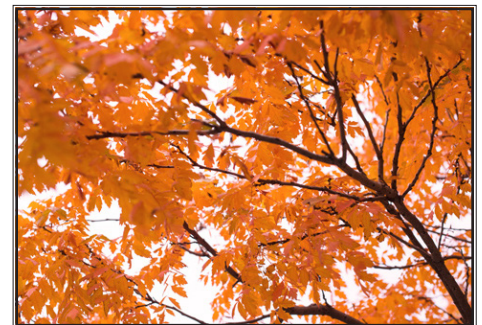
Choose colored clothespins to correspond to nucleobases and plain clothespins for indels.

Hint:

For ease of distribution and organization, maintain nucleobase card decks with the following composition:
A (5) T (15) G (7) C (25)

Each youth group should receive a number of decks equivalent to the number of species they are aligning (e.g., two species to be aligned = two decks with the above card composition).

In addition, each youth group should also have one deck of thirty indel cards.



Keep in mind:

Various factors, such as unequal cross over during meiosis, contribute to gaps being rather common.

However, an optimal sequence alignment typically minimizes the number of gaps and, thus, penalizing for gaps, helps achieve optimality.

Activity 4: A tree is only as strong as its roots: An introduction to gene sequence alignment



(a) **Constant:** This is perhaps the simplest type of gap penalty scoring. In this approach, every gap receives a fixed penalty, regardless of the size of the gap. The total score for a given alignment is thus the number of matches plus the number of gaps.

Assuming a match score of 1 and a gap penalty of -1, the score for the example alignment below would be the number of matches (4; bold) plus the number of gaps (-3), a total of 1.

Species	Aligned sequence
Species 1	A A G T A C - -
Species 2	A - G T - C A A

Under the same scoring assumptions, the score for the example alignment below would be the number of matches (4; bold) plus the number of gaps (-5), a total of -1.

Species	Aligned sequence
Species 1	A A - G T A C - - -
Species 2	A - - G T - C - - A A

Thus, under the Constant gap penalty approach, the optimal alignment would be the former of the two examples above (total score = 1).

(b) **Linear:** This gap penalty scoring approach is a step up in complexity from the Constant approach, in that it takes into consideration each gap length. In this approach, every indel in a gap receives a fixed penalty. The total score for a given alignment is thus the number of matches plus the number of individual indels.

Assuming a match score of 1 and an indel penalty of -1, the score for the example alignment below would be the number of matches (4; bold) plus the number of indels in the gaps (-4), a total of 0.

Species	Aligned sequence
Species 1	A A G T A C - -
Species 2	A - G T - C A A

Under the same scoring assumptions, the score for the example alignment below would be the number of matches (4; bold) plus the number of indels in the gaps (-10), a total of -6.

Species	Aligned sequence
Species 1	A A - G T A C - - - -
Species 2	A - - G T - C - - A A

Thus, under the Linear gap penalty approach, the optimal alignment would be the former of the two examples above (total score = 0).

(c) **Affine:** In this rather widely approach, a heavy penalty is given for what is called a gap opening (the creation of a gap), but gap length is also taken into consideration. The formulaic version of this approach is:

Overall gap penalty = gap opening penalty + gap extension penalty (gap length - 1)

Considering that there are three gaps in the example below and assuming a match score of 1, a gap opening penalty of 5, a gap extension penalty coefficient of 0.1, the score for the example alignment would be calculated as follows:

$$\begin{aligned} \text{Score} &= \text{Matches} - \text{Overall gap penalty 1} - \text{Overall gap penalty 2} - \text{Overall gap penalty 3} \\ \text{Score} &= 4 - (5+0.1(1-1)) - (5+0.1(1-1)) - (5+0.1(2-1)) \\ \text{Score} &= 4 - 5 - 5 - 5.1 \\ \text{Score} &= -11.1 \end{aligned}$$

Species	Aligned sequence
Species 1	A A G T A C - -
Species 2	A - G T - C A A

Under the same scoring assumptions, the score for the example alignment below would be:

$$\begin{aligned} \text{Score} &= \text{Matches} - \text{Overall gap penalty 1} - \text{Overall gap penalty 2} - \text{Overall gap penalty 3} - \text{Overall gap penalty 4} - \text{Overall gap penalty 5} \\ \text{Score} &= 4 - (5+0.1(1-1)) - (5+0.1(1-1)) - (5+0.1(2-1)) - (5+0.1(2-1)) - (5+0.1(4-1)) \\ \text{Score} &= 4 - 5 - 5 - 5.1 - 5.1 - 5.3 \\ \text{Score} &= -21.5 \end{aligned}$$

Species	Aligned sequence
Species 1	A A - G T A C - - - -
Species 2	A - - G T - C - - A A

Thus, under the Affine gap penalty approach, the optimal alignment would be the former of the two examples above (total score = -11.1).

Activity 4: A tree is only as strong as its roots: An introduction to gene sequence alignment



(4) Having shown the youth the three scoring approaches above, facilitators can now challenge the groups to come up with an optimal alignment based on one of these scoring approaches (choice is up to the facilitator, but for time efficiency, the Linear approach is recommended).

Youth should realign their sequences and score them, writing on the board their team name, number of transitions, number of transversions, and alignment score. The alignment(s) with the winning score(s) should be visited by the entire class and the winning team(s) should briefly present their strategy for making the alignment.

(5) As a conclusion, the facilitator should project the two phylogenies depicted in Fig. 4.5 A and B. These phylogenies include the oak species covered in the previous activities, as well as the Asian outgroup (*Quercus glauca*).

The sequences are derived from the nuclear ribosomal internally transcribed spacer (ITS) and have been aligned using two different algorithms: ClustalW and MUSCLE. Youth should be asked to note any differences (the topologies are clearly not the same).

Questions to prompt discussion include:

Which species are depicted as most closely related to one another in Fig 4.5A?

Which species in Fig. 4.5B? Are they the same species?

To which species are they next most closely related?

The answer here is that the algorithm used in alignment can have a profound influence on the outcome of phylogenetic reconstruction. It is thus imperative that workers in this field continue to utilize the most advanced alignment algorithms, which today incorporate sophisticated models of gene evolution.

Talk it over

Share

Describe your strategy for manually aligning the sequence data set?

Did you start at the beginning of one sequence and then proceed systematically in one direction?

Did you look for segments with high matches and then proceed outward from those?

Reflect

Is there a relationship between the best scoring alignment and the number of transitions/transversions or gaps?

Why or why not?

Why is sequence alignment important prior to conducting a phylogenetic analysis?

Which species are the most closely related?

Which ones are the most distantly related?

How did your phylogeny differ from the phenogram developed in Activity 3?

If there were no differences, does that mean you can interpret phenograms as diagrammatic of evolutionary relationships?

Why or why not?

In what types of situations might you expect differences?

Generalize

How can a phylogenetic tree be used in classification?

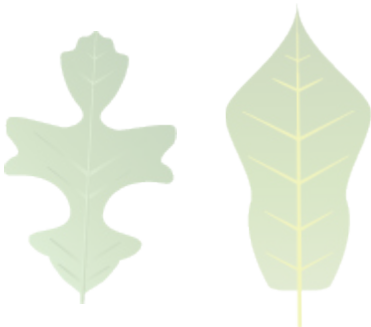
What are its advantages compared to a phenogram?

Apply

What are the disadvantages of basing a classification on phylogenetic relationships?

Note:

Classifications based on phylogenetic relationships have the advantage of being generally predictive (i.e., group membership may predict common properties). Classifications based on morphological similarities generally have the advantage of making it easier to learn and discern between taxa.



Activity 4: A tree is only as strong as its roots: An introduction to gene sequence alignment



Term and Concept Discovery* –

Bayesian analysis: being, relating to, or involving statistical methods that assign probabilities or distribution to events (as rain tomorrow) or parameters (as a population mean) based on experience or best guesses before experimentation and data collection and that apply Bayes' theorem to revise the probabilities and distributions after obtaining experimental data.

Cladogram: a branching diagrammatic tree used in cladistic classification to illustrate phylogenetic relationships.

Classification: systematic arrangement in groups or categories according to established criteria.

GenBank: an online database of publicly available DNA sequences, hosted by the National Institutes of Health (<http://www.ncbi.nlm.nih.gov/genbank/>).

Maximum likelihood: a statistical method for estimating population parameters (as the mean and variance) from sample data that selects as estimates those parameter values maximizing the probability of obtaining the observed data.

Parsimony: the principle that holds that the shortest solution to a problem is the optimal one.

Phenetic: of or relating to taxonomic analysis that emphasizes the overall similarities of characteristics among biological taxa without regard to phylogenetic relationships.

Phenogram: a branching diagrammatic tree depicting similarity relationships.

Phylogeny: a branching diagrammatic tree depicting evolutionary relationships.

Taxonomy: orderly classification of organisms.

*Definitions derived from: Merriam-Webster (2014).

Learn More:

FIND OUT MORE ABOUT GENBANK,
THE NATIONAL INSTITUTES OF HEALTH'S
GENETIC SEQUENCE DATABASE:
<https://www.ncbi.nlm.nih.gov/genbank/>



Photo credit: ** RCB ** via [Foter.com](https://www.foter.com/) / [CC BY 2.0](https://creativecommons.org/licenses/by/2.0/)

Activity 4: A tree is only as strong as its roots: An introduction to gene sequence alignment

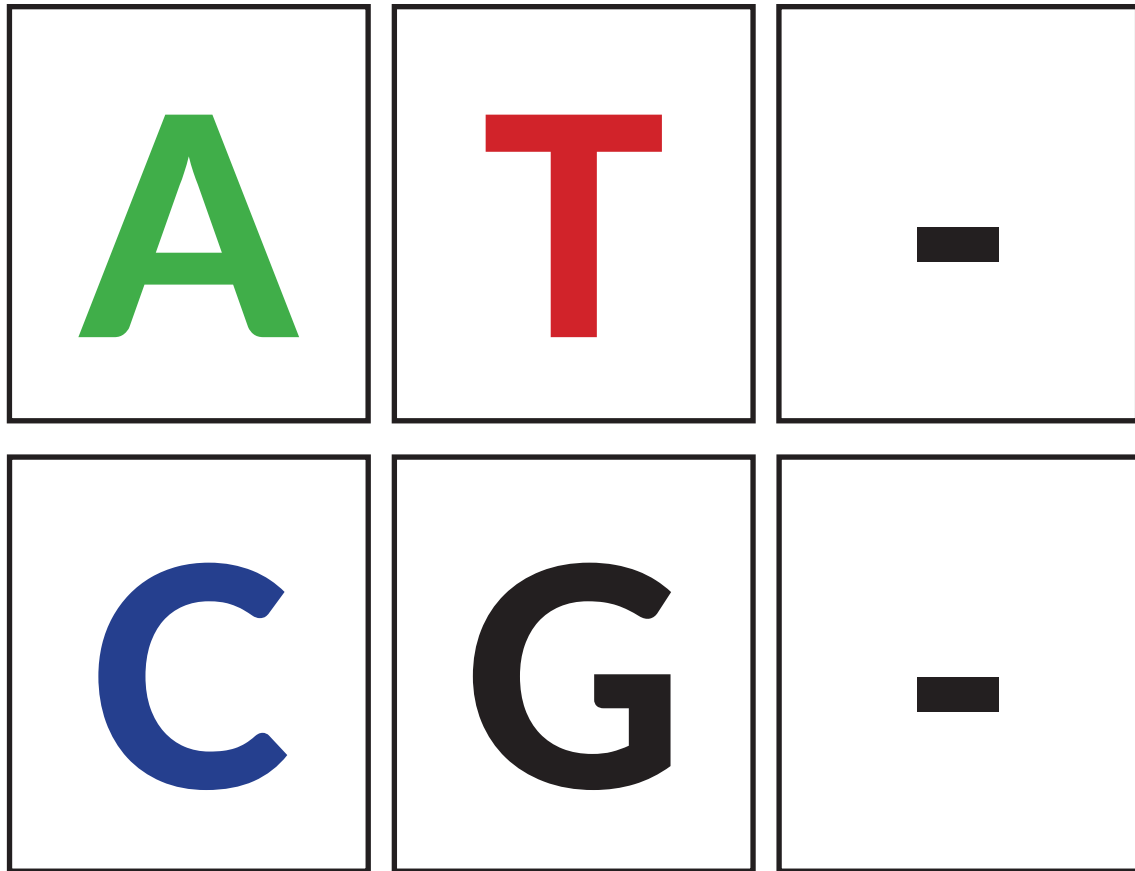


Fig. 4.2. Nucleobase cards. Actual cards are 3.5 x 2.5 inches and available from the project website (along with a "hyphen" card, representing indels).



Fig. 4.3. Sample sequences and alignments using alternative materials for Activity 4: Pipecleaners and mini clothespins. To align the sequence of Species 2 to that of the other species, indels are required in position three and four.

Activity 4: A tree is only as strong as its roots: An introduction to gene sequence alignment

<i>Q. alba</i>	CCCTCGCCCCCTCCTGCGCC - AA ACTCCTTTTACCCTGCGACC - - - T
<i>Q. marilandica</i>	CCCTCGCCCC - CCTGCGC - - AA ACTCCTCTTGCCCTCGTTCCGCAT
<i>Q. montana</i>	CC - TCGCCCCCTCCTGCGC - - AA ACTCCTTTTACCTTGCGACC - - - T
<i>Q. nigra</i>	CCCTCGCCCC - CCTGCGC - - AA ACTCCTCTTGCCCT - CGATCGCAT
<i>Q. stellata</i>	CCCTCGCCCCCTCCTGCGC - - AA ACTTCTTTTACCTTGCGACC - - - T

Fig. 4.4. Sample alignment of select segments of the nuclear ribosomal internally transcribed spacer (ITS) region of four oak species (*Quercus*) using ClustalW. Highlighted bases represent putative transitions (substitution of a purine for a purine, or pyrimidine for a pyrimidine; yellow) and transversions (substitution of a purine for a pyrimidine or vice versa; blue).

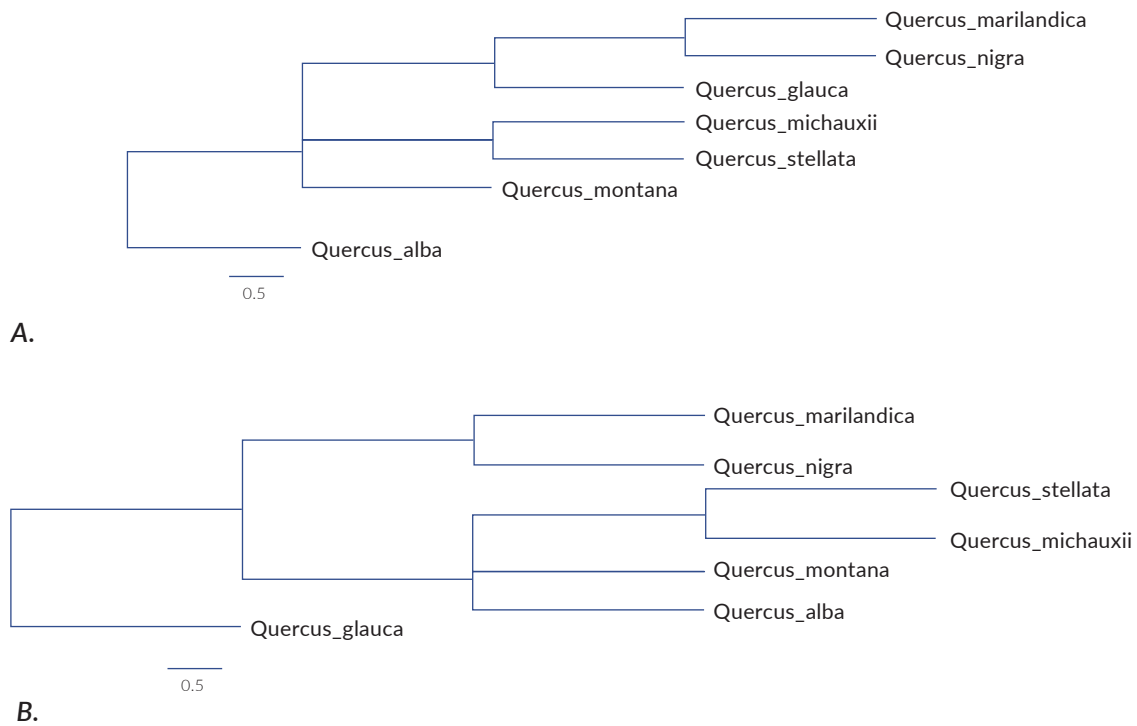


Fig. 4.5. Phylogenies of select oak species based on the nuclear ribosomal internally transcribed spacer (ITS): A. Phylogeny based on ClustalW alignment. B. Phylogeny based on MUSCLE alignment. Phylogenies were generated at phylogeny.fr using TNT (sectorial search, tree fusing).

Clustal is one of the earliest alignment programs, published by Higgins and Sharp in 1988. The package has gone through several refinements since its initial debut (e.g. ClustalV, ClustalW, ClustalX). In part due to its speed, it became widely used, although its performance now ranks among the less accurate of modern programs.

Published by Edgar in 2004, MUSCLE now ranks among the most widely used alignment programs. It provides good accuracy and relatively fast computational speeds.

Activity 5: The changing nature of classification systems

Skill Level: Beginner+, ages 14–18

Learner Outcomes:

- (1) Describe the advantages and disadvantages of different classification systems
- (2) Apply theory and critical thinking to develop new classification
- (3) Defend logically a new classification based on its objectives

Education Standard(s):

Bio.3.5.1 Explain the historical development and changing nature of classification systems

Success Indicators: Describes the advantages and disadvantages of such diverse classification systems as produced by Linnaeus, Bergeret, and the Angiosperm Phylogeny Group, applies theory to develop new classification, able to logically defend classifications

Life Skills: Teamwork, problem solving, decision-making, critical thinking

Tags: Taxonomy, evolution, classification, nomenclature

Time Needed (to complete the activity): 60 min

Materials List:

Paper, writing implements (pencils, pens, or markers), posterboard or powerpoint

Space (setting needed to conduct the activity): Space for several small working groups

Suggested Group Size: 2 or 4 members per group

Introduction:

Classification

Classification is essentially the grouping of things (whether inanimate or living) based upon some organizing criteria. Classifications have no doubt been important throughout human history as a means of not only organizing information, but also providing at least some power of prediction. If one is aware that certain plants are poisonous to the touch, then a classification that allows this information to be communicated and extended to similar or related plants might be quite helpful. For example, if you are severely allergic to poison ivy, you might wish to avoid contact with related species. Knowing that poison ivy belongs to the family Anacardiaceae, thus would allow you to quickly hone in on species that could potentially cause you trouble. Without a classification, you would have little guide to what other species might be of concern.

Linnaeus

Over the course of human history, numerous organismal classifications have been erected, modified, and abandoned, in response to increased understanding and needs. As it is difficult for a classification to meet all possible user needs, there have been (and continue to be) multiple organizing principles. For example, in his *Species Plantarum* (1753), Carl Linnaeus put forth a decidedly pragmatic classification system. His system was artificial (i.e., not based on perceived relationships), but efficiently allowed one to determine species based on plant characters. Species were not ordered according to a "natural" classification (based on perceived relationships or place in the "scala naturae"), but by morphology, particularly number of carpels and stamens. Thus, on one level, *Species Plantarum* functioned much as a Peterson's guide would today.

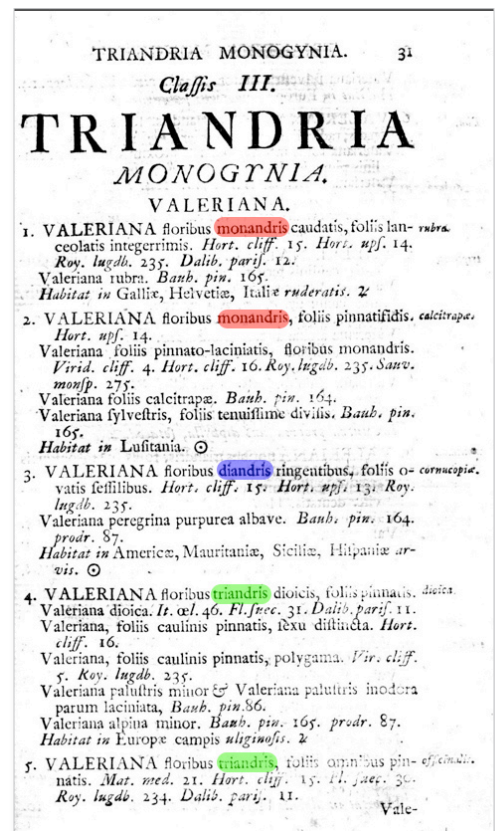


Fig. 5.1. Excerpt from Linnaeus' treatment of *Valeriana* in his *Species Plantarum* (1753), showing grouping of species with a single, two, or three anthers all within his Three-anther class (Triandria). Courtesy of the Peter H. Raven Library, Missouri Botanical Garden.

Activity 5: The changing nature of classification systems

However, although Linnaeus' system facilitated identification, it was not without its problems in application—in large part because Linnaeus clearly recognized the relationship of plants and incompletely applied his artificial criteria. Linnaeus preferred to list congeners together, even when some species differed distinctly in number of parts. Thus, under the section "Triandria" (tri: three; andria: anthers), one can find species of *Valeriana* exhibiting one, two, and three anthers. If one was simply trying to figure out a species with one anther, but didn't know the genus to begin with, one somehow had to divine that others in the genus had three anthers to get to the right section (Fig. 5.1).

Bergeret

Another interesting example of a classification is Jean-Paul Bergeret's three volume *Phytonomatotechnie universelle* (1783–1784), enticingly subtitled: *c'est-à-dire l'Art de donner aux plantes des noms tirés de leurs caractères, nouveau système au moyen duquel on peut de soi-même, sans le secours d'aucun livre, nommer toutes les plantes qui croissent sur la surface de notre globe* [that is to say, the art of giving plants names based on their characters, a new system whereby one can, without help from any book, name all plants that grow on the surface of our globe].



Fig. 5.2. Illustration of snapdragon (*Antirrhinum majus*) from Bergeret's *Phytonomatotechnie universelle* (1873–1874). Bergeret's name is printed in the top left corner (SEFPIAGOADYZE) and the Linnean name in the bottom right (*Antirrhinum majus*). Courtesy of Biblioteca Complutense, Universidad Complutense de Madrid.

Based on such a promising title, one could not be faulted for concluding that such a system might very well represent the end all of all classifications! What did it entail? Like Linnaeus's system before, Bergeret's system focused on plant characters. However, instead of merely using characters to find the correct name for a plant, he used the characters to replace the name itself. Thus, instead of a Latin binomial, each plant species name would be comprised of a sequence of four to fifteen letters, each letter based on an aspect of the plants morphology. Thus, potato (known at his time [and still in ours] as *Solanum tuberosum*) became JEQJIABIAJISBEZ and snap dragon (*Antirrhinum majus*) became SEFPIAGOADYZE (Fig. 5.2)!

Natural classifications

The period that followed Linnaeus and Bergeret was one filled with attempts at abandoning artificial classifications for what became known as natural classifications.

In natural classifications, taxonomists attempted to organize organismal diversity by their perceptions of natural relationships (or relatedness).

Although many of these attempts failed overall to various degrees, a number did in fact capture at least some relationships recognized well into modern times. Antoine-Laurent Jussieu for example (nephew of Bernard Jussieu, who had been Bergeret's mentor) recognized the distinction of monocots and dicots (Fig. 5.3), a relationship accepted until the 20th century.

INDEX METHODI		
ORDINES NATURALES COMPLECTENTIS.		
Acoryloides.		Clas. I
Monocoryloides.	Stamina hypogyna.	II
	perigyna.	III
	epigyna.	IV
Apetale.	Stamina epigyna.	V
	perigyna.	VI
	hypogyna.	VII
Dicoryloides.	Corolla hypogyna.	VIII
	perigyna.	IX
	epigyna.	X
Polypetale.	Stamina epigyna.	XII
	hypogyna.	XIII
Dichlines irregulares.	perigyna.	XIV
		XV

Fig. 5.3. Classification scheme from Antoine-Laurent Jussieu's *Genera Plantarum* (1824). Courtesy of the Peter H. Raven Library, Missouri Botanical Garden

Activity 5: The changing nature of classification systems

Natural Classifications Continued

Unfortunately, he also erroneously thought that spurges, oaks, hazelnuts, nettles, and gourds, were more closely related to pines than other plant families. Jussieu made these determinations in part based upon his interpretation of perceived similarities in inflorescences. Based upon subsequent anatomic and genetic studies, we of course now know that pines (and the other cone bearing plants commonly referred to as gymnosperms) are in fact quite different anatomically and genetically from flowering plants and constitute a distinct evolutionary lineage in their own right.

Genetic Studies:

Technological advances in areas such as microscopy, and, more recently, genetic analysis, are primarily responsible for our changing understanding of the relationship of organisms, and thus the continually changing nature of classifications.

Modern phylogenetic classification

In addition to benefitting from increased morphological knowledge gained from advances in microscopy, our modern understanding of plant relationships also has been dramatically improved by technological advances that allow the extraction and analysis of plant genes. In part because of large, multi-institutional collaborations, we now have a fairly clear picture of at least higher-level relationships among flowering plants.

One of the major contributors to this effort has been the Angiosperm Phylogeny Group (APG 2009). This group is comprised of numerous individual contributors at many institutions, working together to provide and analyze genetic sequences of plants. The result of this collaboration has been a series of progressively refined classifications, known in the taxonomic community as APG I, APG II, and APG III (Fig. 5.4). As the majority of higher-level relationships is believed to be stable, many herbaria have begun reorganizing their flowering plant collections according to a linear sequence of families derived from APG III (Haston et al. 2009). However, this is not to say that the tree of plant life is completely understood. In fact, although higher-level relationships are much better understood, there remain significant gaps in our understanding of relationships and circumscription below the family level. In addition, there remain issues basic to any classification that ultimately can be reduced to the objective one sees as most important for a classification. (Continued on page 52)

Term and Concept Discovery* –

Artificial classification: a classification based on rules other than evolutionary relationships.

Monophyletic: developed from a single common ancestral form; a clade including the most recent common ancestor and all of its descendants.

Monotypic: including a single representative – used especially of a genus with only one species.

Natural classification: a classification based on perceived relationships rather than artificial categorization.

Nomenclature: the rules and processes of naming organisms.

Phylogenetic classification: a classification based on evolutionary relationships.

Predictive classification: a classification based on the objective of forecasting characteristics based on group membership.

*Definitions derived from: Merriam-Webster (2014).



Activity 5: The changing nature of classification systems

Learn More:

TO LEARN MORE ABOUT ANGIOSPERM CLASSIFICATION, SEE THE ANGIOSPERM PHYLOGENY WEBSITE: <http://mobot.org/MOBOT/research/APweb/>

TO EXPLORE HISTORICAL BIOLOGICAL LITERATURE, SEE THE BIODIVERSITY HERITAGE LIBRARY: <http://www.biodiversitylibrary.org>

Note that the full classification of organisms includes not just the scientific name (which is the name at the rank of species or below), but additional higher level categories, including, but not limited to, family, order, subclass, and kingdom. For *Quercus alba*, such a classification would include:

- Kingdom - Plantae
- Division - Magnoliophyta
- Subclass - Hamamelidae
- Order - Fagales
- Family - Fagaceae
- Genus - *Quercus*
- Species - *Quercus alba*

The circumscription and recognition of the various ranks continues to be refined, as our knowledge of evolutionary relationships advances.

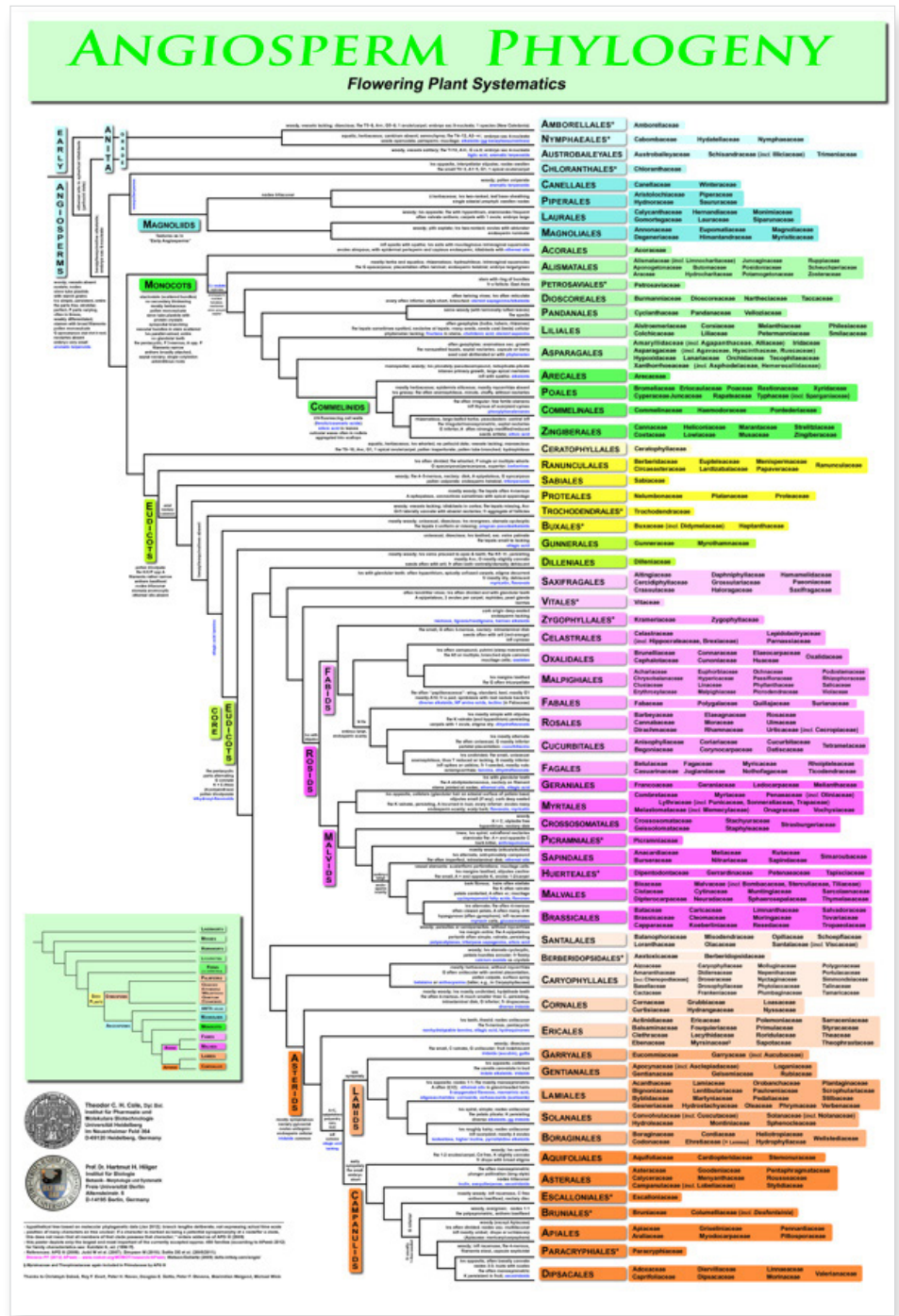


Fig. 5.4. Flowering plant (angiosperm) tree of life derived from APG III.

Poster courtesy of: Cole TCH, Hilger HH. (2016) Angiosperm Phylogeny Poster – Flowering Plant Systematics. PeerJ Preprints 4:e2320v1 <https://doi.org/10.7287/peerj.preprints.2320v1>, CC BY 4.0

Activity 5: The changing nature of classification systems

Modern phylogenetic classification continued

Consider the relationship of the three species A, B, and C in Fig. 5.5. Note that species B is considerably derived, differing from A by a number of character states (indicated by the black rectangles). There are a number of ways this relationship could be classified. One could: (a) consider all species monotypic and thus give each their own genus (Fig. 5.5a), (b) consider them all in one genus (Fig. 5.5b), (c) consider A and B in the same genus, but place C in a separate genus (Fig. 5.5c), (d) consider A and C in the same genus, but place B in a separate genus (Fig. 5.5d). Each approach has its advantages and disadvantages. An approach that lumps B into any other genus aside from its own essentially disregards the significant evolution undergone by the taxon and dilutes the ability to employ its morphological divergence as an identification aid. However, recognizing B in its own genus and A/C in another, results in the odd situation in which one species in a genus (i.e., A) is more closely related to another genus (B), than to its congener (C). Such a situation would violate the principle of monophyly, which has become perhaps the dominant classification criterion over the past few decades in plant taxonomy. A group exhibits monophyly if all descendants of the most recent common ancestor are included. Classifying species into higher-level groups (e.g., genera, families, etc.) based on this principle optimizes the predictivity of evolutionary relationships from taxon names themselves.

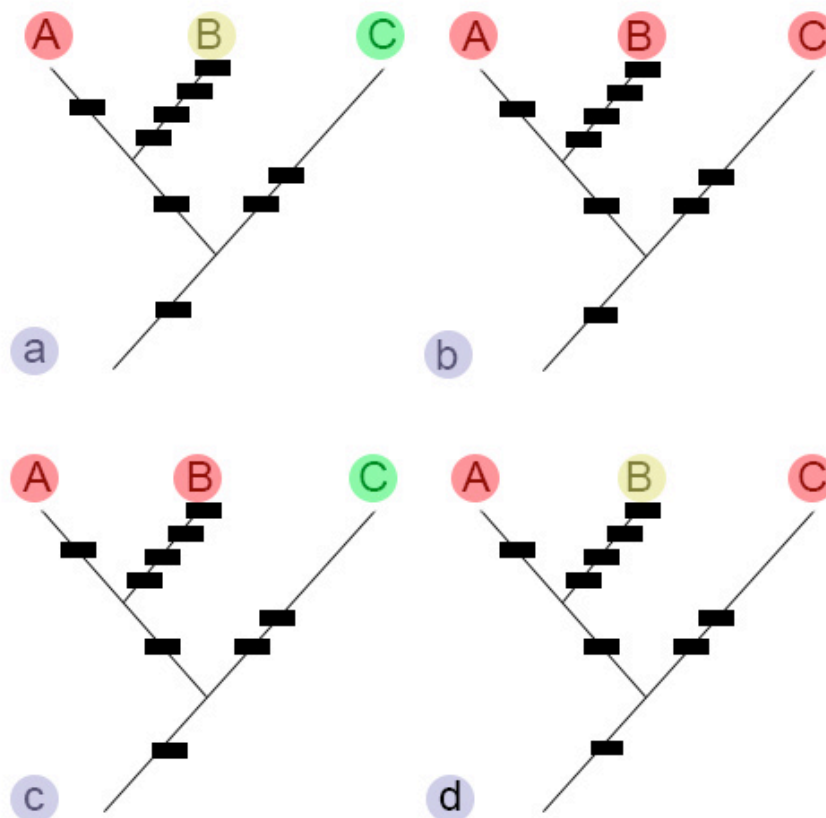


Fig. 5.5. Relationship of three species (A, B, and C) that could be classified in numerous ways, including: (a) consider all species monotypic and thus give each their own genus, (b) consider them all in one genus, (c) consider A and B in the same genus, but place C in a separate genus, (d) consider A and C in the same genus, but place B in a separate genus. Black rectangles represent character states that have diverged.

Activity 5: The changing nature of classification systems

Background information:

In this activity, youth will have the opportunity to take their hands-on experience from the previous activities (identifying previously known species, discovering and naming new species, analyzing their relationships from both a morphological and molecular perspective, etc.) to develop their own classification system for the oaks covered in Activities 2, 3, and 4.

This activity can be run as an in-class small group activity or as a homework assignment followed by group presentations.

- Before the Activity (steps facilitator must take prior to the activity): Arrange youth in small groups. Be familiar with different plant taxonomic classifications, such as Bergeret, Linnaeus, and APG III (see supporting materials), in order to facilitate discussions. Ultimately, having worked out various classifications themselves, youth will be better prepared to understand why and how classification systems change.
- Let's Do It! (steps to conduct the activity): Facilitator guides youths through the activity, answering questions as needed. The directive for each youth group is to:

Activity 4:

- (1) Imagine you have the opportunity to classify and name the species you've encountered in Activities 2 through 4 in any way you want. How would you do it? Would your names reflect relationships or morphology or something else? Would you consider morphology, gene sequences, other features?
- (2) Create and present a poster, powerpoint, or video showing your classification, along with brief notes on its usership, advantages, and disadvantages. If making posters, youth may benefit from doing so outside or in a natural setting.
- (3) Defend your classification. On what principles is it based?

Opening Questions:

What do you think should be the objective of a classification?

To be easily memorized?

To be predictive?

Scientists still fine-tune classifications to meet diverse scientific and societal needs.

Talk it over

Share

Describe how you classified the oak species?
What is the underlying principle?
Did you consider morphology, gene sequence data, or other features?

Reflect

What is the purpose or application of your classification?
What audience would be most likely to use it?

Generalize

In what respects is your classification superior or inferior to existing or historical classifications?

Apply

How would you extend your classification to other species?
That is, how can it be scaled to other taxonomic groups?

Activity 6: Keys to the flora

Skill Level: Beginner+, ages 14–18

Learner Outcomes:

- (1) Analyze morphological data
- (2) Develop and employ dichotomous diagnostic keys based on plant morphology
- (3) **Advanced:** Develop new identification guide to trees

Education Standard(s):

North Carolina Essential Standards in Science

Bio.3.5.2 Analyze the classification of organisms according to their evolutionary relationships (including: dichotomous keys and phylogenetic trees)

Success Indicators: Acquires and synthesizes morphological data, able to correctly use and develop dichotomous keys, applies critical thinking to develop new resource

Life Skills: Teamwork, problem solving, decision-making, critical thinking

Tags: Taxonomy, classification, nomenclature, dichotomous keys

Time Needed (to complete the activity): 60 to 90 min, but Option 1 may require additional class sessions

Materials List:

Option 1: Paper, smartphones or desktop computers with access to internet, cameras or smartphones with camera, word processing software, writing implements (pencils or pens).

Option 2: Paper, plant material of ten different species (or species print-outs from project web site), writing implements (pencils or pens)

Space (setting needed to conduct the activity): Space for several small working groups

Suggested Group Size: 2–4 members per group

Introduction:

The perhaps most practical (or at least most widely used) culmination of taxonomic work, the result of countless hours of field work, studying specimens, working in the lab or herbarium, analyzing variation and relationships (genetic or morphological), etc., is the taxonomic guide. Taxonomic guides provide a pragmatic summary of species to facilitate the application of names by anyone in any discipline. Depending on their objective and audience, taxonomic guides can variously include species descriptions, images, tools for distinguishing species (such as artificial keys or arrangements; **Figs. 6.1 & 6.2**), etc.

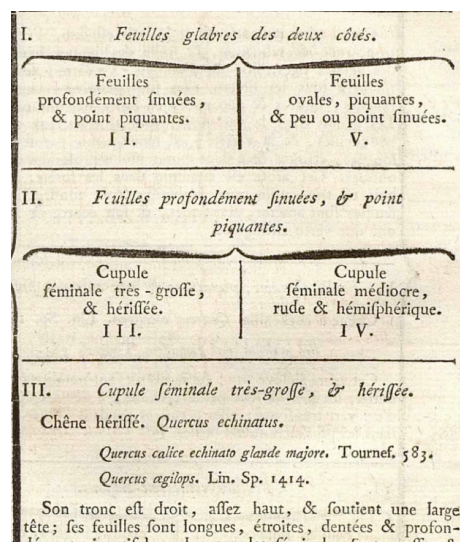


Fig. 6.1. Excerpt of oak treatment from *Flore française* (Lamarck 1779), perhaps the first flora to introduce analytic identification keys. Courtesy of Biblioteca Digital del Real Jardín Botánico de Madrid.

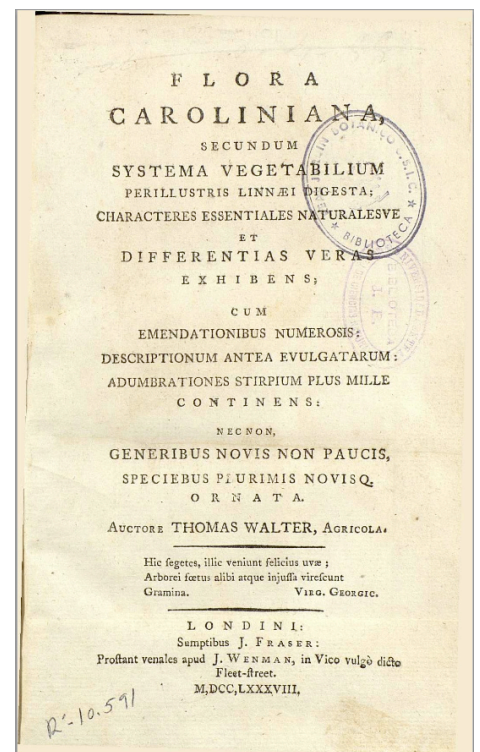


Fig. 6.2. Cover page of *Flora Caroliniana* (Walter 1788), the first flora of the Carolinas. Courtesy of Biblioteca Digital del Real Jardín Botánico de Madrid.

The most comprehensive and scientific guides are variously known as monographs or revisions. More popular works are derivative, frequently including less species or less data, and are commonly known as field guides. There is a considerable spectrum between monographs and field guides/apps, variously tailored in content to a specific audience.

Taxonomic guides to the plants of a specific region are often referred to as “floras” (or also “florulas”, if making an arbitrary distinction in the extent of area treated). Some floras are essentially checklists of the species of a given area, including as well discussions of site aspects such as climate, geology, soils, and plant communities. Others take things a step further and provide their users not just species names, but means to distinguish species (usually dichotomous keys; **Figs. 6.3 & 6.4**).

Activity 6: Keys to the flora

For scientific purposes, voucher specimens are archived for each species encountered in the study of an area. These specimens are deposited in a herbarium and thus available for further research (including verification by others). However, interest has been growing in using photography to document species and discussions have begun about what standards should be required (Baskauf & Kirchoff 2008).

While photographs should never replace specimens in a scientific flora, they can be very useful additions. For photographs to be of most use, it is important that they reflect details of the morphology of each plant that are crucial to its identification. As beautiful as our native sunflowers (*Helianthus* spp., Asteraceae) are for instance, pictures of just their flowering heads are essentially useless for identification. Much more important would be photographs of the backsides of the rays, the involucre bracts, and the backsides of the leaves (to demonstrate if leaves are white waxy or green, glandular or eglandular, pubescent or hairless, etc.).

Floras and field guides are of very practical importance, as they are usually the most accessible taxonomic product for non-taxonomists.

Imagine being a forester and in charge of managing a forest for timber, wildlife, or recreational value. Your task would be impossible to carry out competently without an understanding of the plant communities and species on your property. A flora or field guide is your key to learning these basics (whether in college or later on the job).

Imagine you are an entomologist trying to understand reasons behind declining numbers of a certain pollinator. Among your first tasks might be to learn the plants visited by the pollinator. What will help you? A flora or field guide!

Imagine you are a turf manager for a successful golf course. To maintain the turf in prime condition will require identification of weeds prior to making a decision about treatment. As herbicide response differs among weeds, making the proper determination (using a flora or field guide, of course!) will save you time, effort, and money.

Imagine you raise horses and want to make a new pasture available. Proper identification of the plants prior to introducing horses could go a long way to avoiding poisoning.

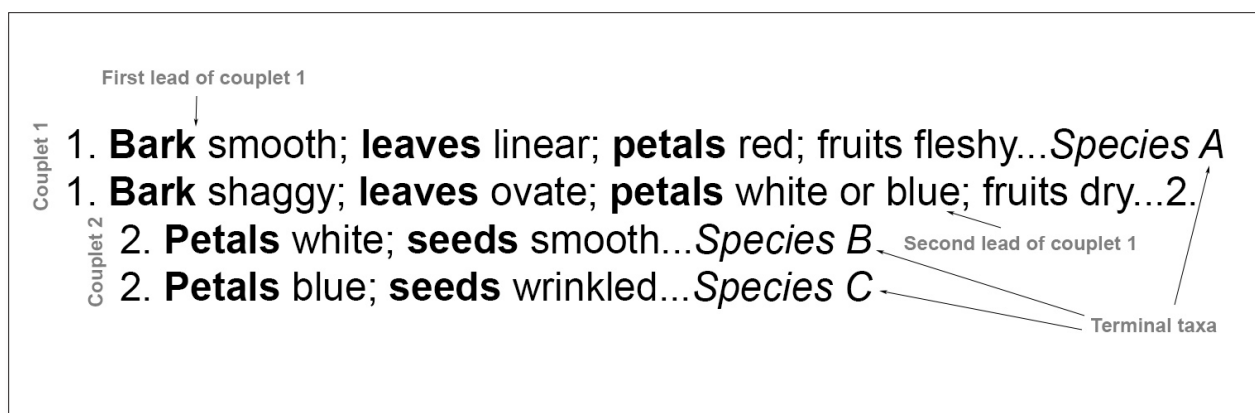


Fig. 6.3. Anatomy of a dichotomous key. Dichotomous keys are composed of two contrasting statements ("leads"), these together referred to as "couplets". The terminal units can be of any taxonomic rank (e.g., order, family, genus, species, subspecies, etc.), depending on the objective of the key. Terminal taxa (species in the above excerpt) in terminal couplets are typically arranged alphabetically. Characters in each lead must be parallel and contrasting.

Activity 6: Keys to the flora

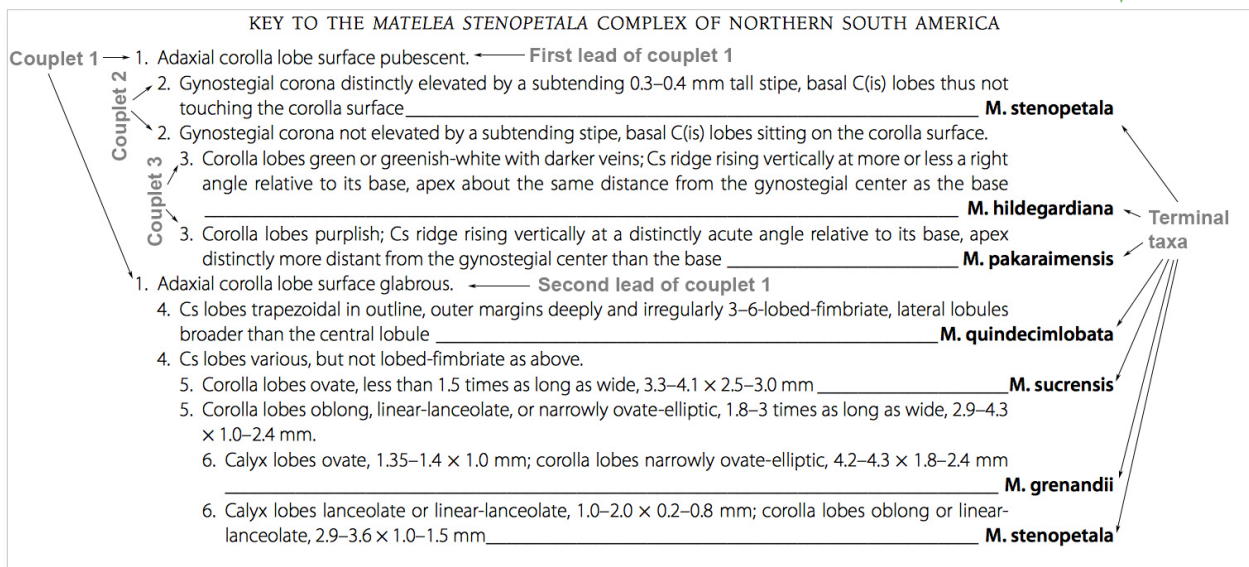


Fig. 6.4. Sample dichotomous key (based on Krings 2011). Note the alphabetical arrangement of terminal taxa in terminal couplets.

Opening Questions:

What tree species are found at your school or around your home?

How can local floras (or guidebooks) be useful to management, research, and commerce?

Scientists continue to produce site-specific floras to support a variety of needs, from applied management to fundamental research.



Background Information:

The objective of this activity is for youth to learn to understand and properly construct dichotomous identification keys. The activity can be carried out in a number of ways, depending on the time available and level of the youth.

Two options are provided below, that can be adapted as needed:

- (1) Option 1: Developing a guide to the trees of the school grounds or facilitator chosen area.
[time intensive, likely requiring more than one session]

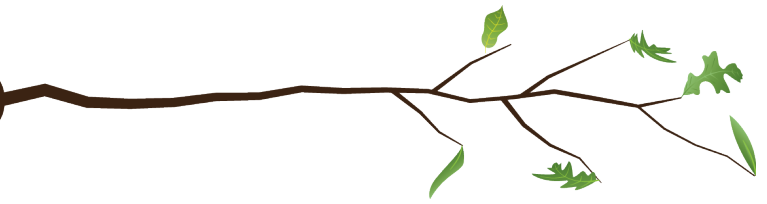
and

- (2) Option 2: Developing dichotomous keys from facilitator provided live plant material or images from select species as available from the project web site.
[time minimal to moderate, 60 to 90 min, depending on the amount of plant material and level of youth]



Fig. 6.5. Trees of North Carolina: An online and mobile app (TNCWG 2014).
<http://herbarium.ncsu.edu/tnc>

Activity 6: Keys to the flora



Activity 6: Option 1

Working in teams, youth will employ the richly illustrated dichotomous keys from a recently developed web app at North Carolina State University to identify trees on school grounds. Once a list of species has been compiled, youth will develop their own dichotomous keys to facilitate the identification of school trees. Keys will be bundled into a comprehensive guide and can be supplemented with photographs taken by youth or from public domain web sources.

- Before the Activity (steps facilitator must take prior to the activity): Arrange youth in small groups. Each group will be responsible for the trees of a specific sector of the school grounds.
- Let's Do It! (steps to conduct the activity): Facilitator guides youth through the activity, answering questions as needed. The directive for each youth group is to:

- (1) Now is your chance to make your own guidebook to the trees of your school or facilitator chosen area. Work with your instructor to divide into groups, each with responsibility for a specific sector of your chosen area.
- (2) Fan out across the area to inventory the trees, taking either clippings, or photographs or both of all species you encounter. Remember, numerous characters are often needed for conclusive identification. Thus, make sure your data gathering is comprehensive. For example, if you are taking clippings of twigs, make note or photograph bark characters, as well. If you are only taking photographs, make sure to capture details of the bark, twigs, buds, leaves (outline, margin details, lower surface details, etc.), and any flowers or fruits as may be available. You can include these in your guide to illustrate each species. If you have chosen a natural area as your focus, it would be useful to make notes on the habitats for each tree, as well. You may wish to identify species in the field or back in the classroom. It would be helpful to study *Trees of North Carolina (Fig. 6.5; TNCWG 2014)* in advance of field work to get an idea of what sort of characters you'll need to capture (e.g., leaves, fruits?).
- (3) Identify or confirm each species using the resources provided.
- (4) Collaboratively develop a comprehensive species list by having each group report on the species list from their particular sector.
- (5) Once you have a finalized species list, make your own dichotomous keys. You can divide the task by having one group write a key to all genera and other groups write keys to the species in each genus.
- (6) Compile all keys in a word document and illustrate with pictures from the *Trees of North Carolina (TNCWG 2014)* or other source.

Talk it over

Share

- Which species did you find in your sector?
- What are the distinguishing characteristics?
- Which trees were familiar?
- Where else have you seen them?
- Which species of those assigned to your group were familiar to you?
- Where else have you seen them?

Reflect

- What aspects of the inventory or identification did you find challenging?
- Why?

Generalize

- What applications can you see from a local flora or guidebook?
- Why is correct identification of organisms such as trees important?

Apply

- What resources would you need to inventory a poorly explored region of the world, where there was no guidebook to help you identify species?

Learn More:

LOOKING FOR MORE IMAGES OF TREES OF NORTH AMERICA? TRY BIOIMAGES:

<http://bioimages.vanderbilt.edu/>

Activity 6: Keys to the flora



Activity 6: Option 2

Working in teams, youth will develop their own dichotomous keys to facilitate the identification of either (1) some common trees of North Carolina, based on images available on the project web site, or (2) plant material provided by the facilitator (at least ten different species recommended). Keys can be supplemented with photographs taken by youth or from public domain web sources, at the discretion of the facilitator.

- Before the Activity (steps facilitator must take prior to the activity): Download images of common tree species from the project web site (or make sure that students can access the site during class), or, obtain live plant material for class. Arrange youth in small groups.
- Let's Do It! (steps to conduct the activity): Facilitator guides youth through the activity, answering questions as needed. The directive for each youth group is to:

(1) Carefully study the provided images or plant material, taking particular note of the morphology. Important characters to pay attention to include whether leaves are alternate or opposite, simple or compound, whether leaf margins are entire, toothed, or scalloped, whether twigs are hairy or not, whether fruits are fleshy or dry, etc. Youth may wish to construct character matrices to help guide the development of dichotomous keys.

(2) Develop dichotomous key(s) to the species provided. If some genera have more than one species, youth might develop more than one key: one to genera and then another to species for those genera with multiple species. Youth should keep in mind that keys need to be dichotomous (i.e., each split is binary), absolutely parallel in terms of characters, and that terminal taxa should be arranged alphabetically.

(3) *Extension:* Depending on the time available and class interest, the facilitator can provide different sets of species to each group and, once all groups have made their keys, have the groups test each other's keys on each other's plant material. The class can discuss which keys worked best and why.

Term and Concept Discovery* –

Dichotomous key: a key for the identification of organisms based on a series of choices between alternative characters.

Flora: a treatise on or list of the plants of an area or period.

Inventory: a complete list of things (e.g., organisms) that are in a place

Classification: systematic arrangement in groups or categories according to established criteria.

*Definitions derived from: Merriam-Webster (2014).

Talk it over

Share

Which species of those assigned to your group were familiar to you?

Where else have you seen them?

Reflect

What aspects of the making the dichotomous keys did you find challenging?

Why?

Generalize

What applications can you see from a local flora or guidebook?

Why is correct identification of organisms such as trees important?

Apply

What resources would you need to inventory a poorly explored region of the world, where there was no guidebook to help you identify species?

References



Activity 1

Alfred Russel Wallace, Photograph, London Stereoscopic & Photographic Company (active 1855-1922) - First published in Borderland Magazine, April 1896 [<https://commons.wikimedia.org/wiki/File:Alfred-Russel-Wallace-c1895.jpg>] Viewed 4 August 2016.

American Kennel Club. 2014. Complete breed list. [<http://www.akc.org>]

Centers for Disease Control. 2013. *Antibiotic resistance threats in the United States, 2013*. Centers for Disease Control, Atlanta. [<http://www.cdc.gov/drugresistance/threat-report-2013/>]

DiGiovanni, N. 1989. *The chips are down: A natural selection simulation*. Evolution & the Nature of Science Institutes. [www.indiana.edu/~ensiweb/]

Barraud, H.R. (1845 - 1896) - *Charles Darwin: The Last Portrait*, Scientific American, November 1995, pp. 78-79 [https://commons.wikimedia.org/wiki/Charles_Darwin#/media/File:Charles_Darwin_photograph_by_Herbert_Rose_Barraud,_1881_2.jpg] Viewed 4 August 2016.

Merriam-Webster. 2014. merriam-webster.com. Merriam-Webster, Inc. [<http://www.merriam-webster.com/dictionary/>]

Norsworthy, J.K., G. Griffith, T. Griffin, M. Bagavathiannan, and E.E. Gbur. 2014. *In-field movement of glyphosate-resistant Palmer amaranth (Amaranthus palmeri) and its impact on cotton lint yield: Evidence supporting a zero-threshold strategy*. Weed Science 62: 237-249.

Activity 2

Davis, J.I. and K.C. Nixon. 1992. *Populations, genetic variation, and the delimitation of phylogenetic species*. Systematic Biology 41: 421-435.

Dcrjrs. *White oak (Quercus alba) acorns* - one prolific tree can nearly cover the ground in a good year. Duke Forest Korstian Division, Durham North Carolina. 15 November 2011 [https://commons.wikimedia.org/wiki/File:White_oak_Quercus_alba_prolific_acorns.jpg] Viewed 19 July 2016.

Gould, J. *Darwin's finches or Galapagos finches*. Darwin. 1845. [https://commons.wikimedia.org/wiki/File:Darwin%27s_finches_by_Gould.jpg] Viewed 19 July 2016.

Haeckel, E. 1866. *Árbol de la vida según Haeckel, E. H. P. A. Generelle Morphologie der Organismen : allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von Charles Darwin reformierte Descendenz-Theorie*. Berlin. [https://commons.wikimedia.org/wiki/File:Haeckel_arbol_bn.png] Viewed 19 July 2016.

O'Brien, J. 2006. *Tyloses in oak*. Laboratory. US Forest Service Northeastern Area archives. [<http://www.forestryimages.org/browse/detail.cfm?imgnum=5040007#collapseseven>] Viewed on 16 July 2016.

Merriam-Webster. 2014. merriam-webster.com. Merriam-Webster, Inc. [<http://www.merriam-webster.com/dictionary/>]

Nixon, K.C. and Q.D. Wheeler. 1990. *An amplification of the phylogenetic species concept*. Cladistics 6: 211-223.

Simpson, S. Sunlight through yellow oak leaves. [<https://unsplash.com/@sophiecool655>] Viewed 15 July 2016.

Trees of North Carolina Working Group (TNCWG). 2014-present. *Trees of North Carolina*. Version 1.0. North Carolina State University, Raleigh. [<http://herbarium.ncsu.edu/tnc/>]

Activity 3

Merriam-Webster. 2014. merriam-webster.com. Merriam-Webster, Inc. [<http://www.merriam-webster.com/dictionary/>]

Mora, C., D.P. Tittensor, S. Adl., A.G.B. Simpson, and B. Worm. 2011. *How many species are there on earth and in the ocean?* PLoS Biol 9(8): e1001127. [<http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001127#abstract0>]

Opperdoes, F. 1997. *Construction of a distance tree using clustering with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA)*. [<http://www.icp.ucl.ac.be/~opperd/private/upgma.html>]

Powers, D. A., and F.J. Rohlf. *A Numerical Taxonomic Study of Caribbean and Hawaiian Reef Coral*. Systematic Zoology 21.1: 53-64. Web. [http://www.jstor.org/stable/2412257?seq=5#page_scan_tab_contents]

References

Activity 4

Couse-Baker, R. 2011. Oak tree photo. [<https://www.flickr.com/photos/29233640@N07/6314049882/>] via [<http://foter.com/>]

Marengo, G.G. Autumn tree photo. [<https://unsplash.com/@gabrielgm>]

Merriam-Webster. 2014. merriam-webster.com. Merriam-Webster, Inc. [<http://www.merriam-webster.com/dictionary/>]

Ridley, M. 2003 *Evolution, third edition*. [<http://www.wiley.com/WileyCDA/WileyTitle/productCd-1405103450.html>]

Activity 5

Bergeret, J.-P. 1873. Illustration of snapdragon. Courtesy of Biblioteca Complutense, Universidad Complutense de Madrid.

Cole, T.C.H. and H.H. Hilger. 2016. Angiosperm Phylogeny Poster, *Flowering Plant Systematics*. PeerJ Preprints 4:e2320v1
<https://peerj.com/preprints/2320v1/>

Haston, E., Richardson, J.E., Stevens, P.F., Chase, M.W., and D.J. Harris. 2009. *The Linear Angiosperm Phylogeny Group (LAPG) III: a linear sequence of the families in APG III*. *Botanical Journal of the Linnean Society* 161: 128–131.

Jussieu, A.-L. 1824. Excerpt from *Genera Plantarum*. Courtesy of Peter H. Raven Library, Missouri Botanical Garden.
<http://www.biodiversitylibrary.org/bibliography/284#/summary>

Linnaeus, C. 1753. *Species Plantarum*. Laurentius Salvius, Stockholm. [<http://biodiversitylibrary.org/page/358012>]

Linnaeus, C. 1753. *Valeriana in Species Plantarum*. Courtesy of the Peter H. Raven Library, Missouri Botanical Garden.

Activity 6

Baskauf, S.J. and B.K. Kirchoff. 2008. *Digital plant images as specimens: Towards standards for photographing living plants*. *Vulpia* 7: 16–30.
http://vulpia.ncsu.edu/pdf/Baskauf_&_Kirchoff_Digital_Plant_Images.pdf

Krings, A. 2011. *Matelea pakaraimensis (Apocynaceae: Asclepiadoideae), a new species in the Matelea stenopetala complex from Guyana*. *Journal of the Botanical Research Institute of Texas* 5: 101–104.

Lamarck, J.-B., de. 1779. *Flore française*. De L'Imprimerie Royale, Paris.

Merriam-Webster. 2014. merriam-webster.com. Merriam-Webster, Inc. [<http://www.merriam-webster.com/dictionary/>]

Trees of North Carolina Working Group (TNCWG). 2014–present. *Trees of North Carolina*. Version 1.0. North Carolina State University, Raleigh.
<http://herbarium.ncsu.edu/tnc/>

Walter, T. 1788. *Flora Caroliniana*. Sumptibus J. Fraser, Prostant venales apud J. Wenman, in Vico vulgo dicto Fleet-street, London.



From Natural Selection
to Classification:
Building on Concepts

Beginner +
Facilitator's Guide

NC STATE UNIVERSITY

