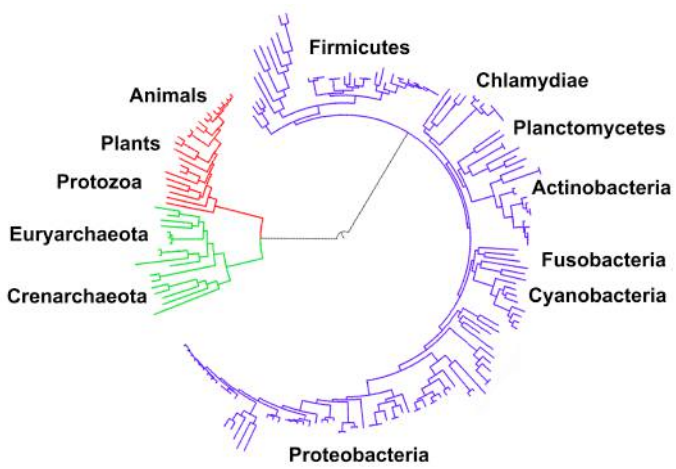


Co-Evolution in the High School Classroom: Constructing and Applying Phylogenies to Intepret Plant and Pollinator Interactions

Jacob Landis: University of Florida, Florida Museum of Natural History

Jennifer Broo: St. Ursula Academy, Cincinatti, OH

Jessica Mahoney: Edgewater High School, Orlando, FL

Julie Bokor: UF Center for Precollegiate Education and Training

Motivation of Module

When I ask students if they like Botany and plants:

Reality

The Hope

Context

- Year One
  - 24 10th grade students (Science Quest program)
  - ~90 11th and 12th grade students (STEM Immersion program)
- Year Two
  - ~90 10th – 12th grade students (STEM Immersion)
  - 10 secondary science teachers
- 6 hour module
- Scaffolds student understanding of phylogenetics

Module: authentic experimental sequence

- Observation of morphological characteristics
- DNA extraction
- DNA amplification (PCR)
- Verification of PCR product (gel electrophoresis)
- Construction of morphological tree
- Construction of molecular tree
- Mapping on traits

Module Activities

- Four main activities:

  1) background information on phylogenetic analyses and importance of botanical knowledge,

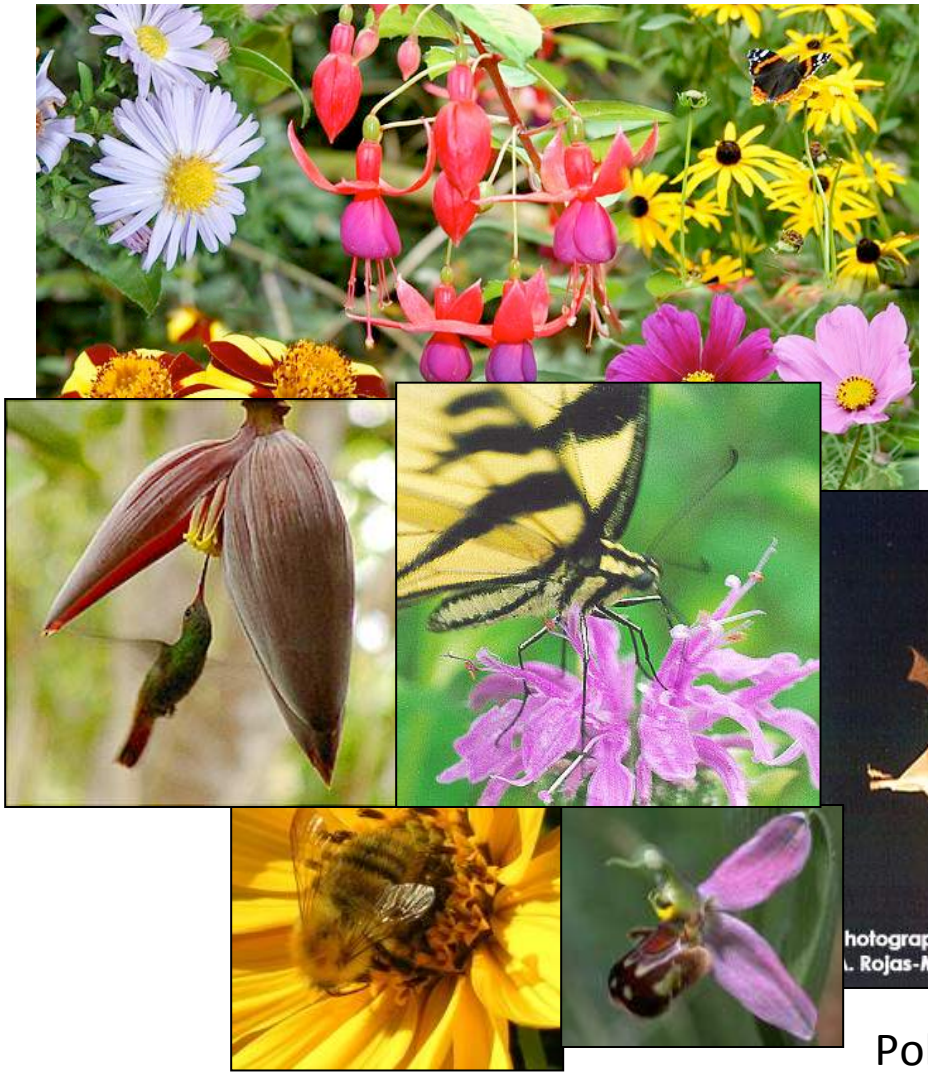
  2) collection of floral morphological features,

  3) common laboratory techniques for generating molecular data, and

  4) phylogenetic analyses involving morphological and molecular data.

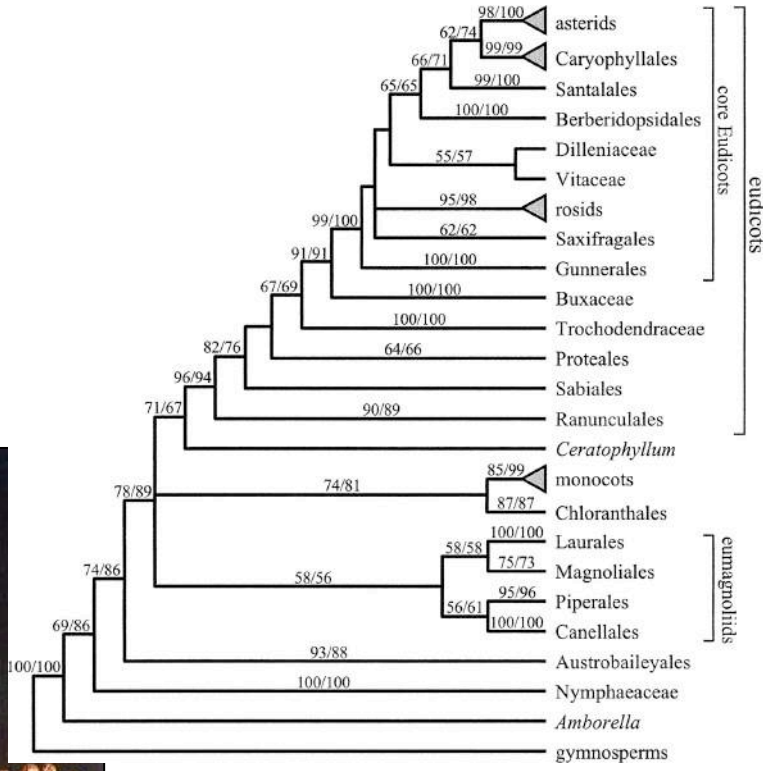
Background

Floral diversity



Pollinator syndromes

Phylogenetics



Floral Morphological Features

PLANT MORPHOLOGY AND DNA EXTRACTION

1. Select four plants for morphological and genetic analysis. Record their names below.

Plant Number	Plant Name	Description of Plant
1		
2		
3		
4		

2. Observe and describe your four plants. As a group, identify floral characters you can use in order to group them by similarity. Which plants are most similar and why? Draw and describe below. Do you think the morphological relationships you describe also indicate genetic relatedness?

Morphological Phylogenetic Analysis

Species	Character 1	Character 2	Character 3
Coding	0 = 1 =	0 = 1 =	0 = 1 =
Maltese Cross			
Nicotiana			
Petunia			
Snapdragon			
California poppy			
Empress of India			
Blue Flax			
Pentas			
Verbena			
Vinca			
Red Phlox			
Salvia			

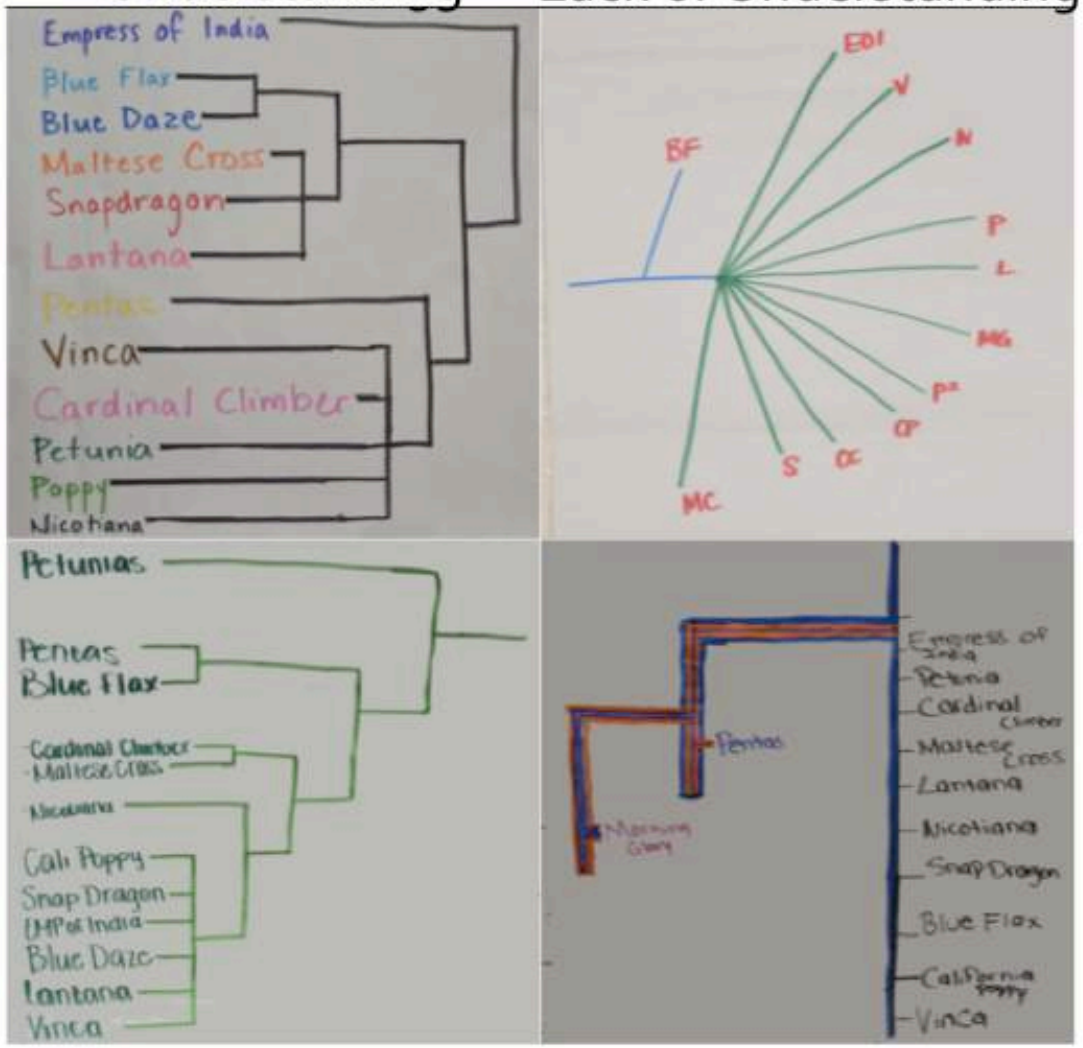
Tubular (or not)

Symmetry (radial or not; bilateral or not)

Morphological Phylogenetic Analysis

Complete/Developing Understanding

Lack of Understanding



DNA extractions

- Label tubes
- Take hole punch of leaf material
- Extraction solution
- Cook at 95°C for 10 minutes
- Dilution solution
- Sigma Extract-N-Amp Kits

Molecular Biology Techniques

1. Identify the following reagents at your student workstation, or collect from a common station:

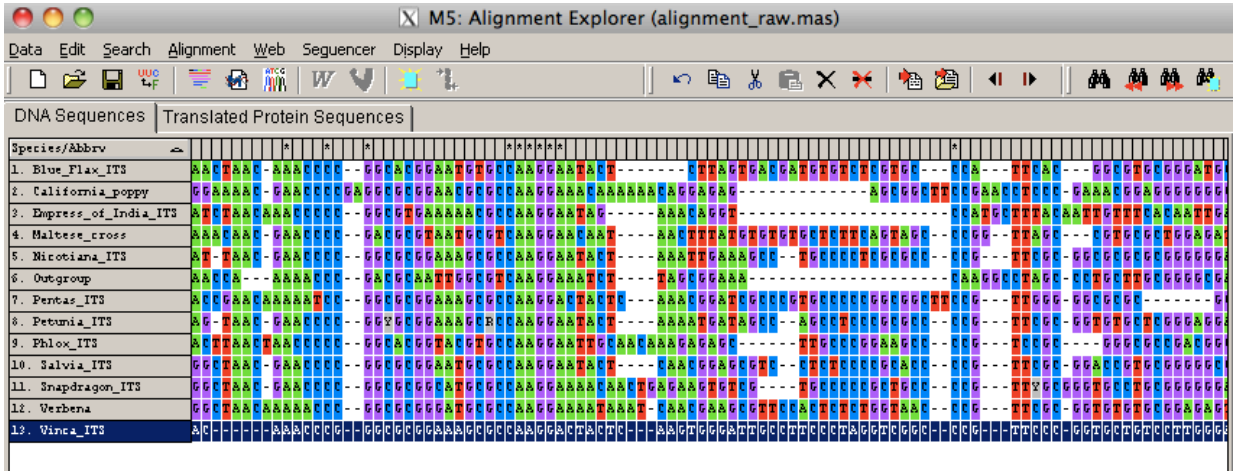
Tube Label	Contents	✔
PCR Mix	REDExtract-N-Amp PCR Ready Mix (Contains $MgCl_2$, dNTPs, Taq Polymerase)	
Water	Sterile water	
Forward	Forward primer	
Reverse	Reverse primer	
Positive	Positive control	
1-4	Your plant DNA samples from the previous procedure	

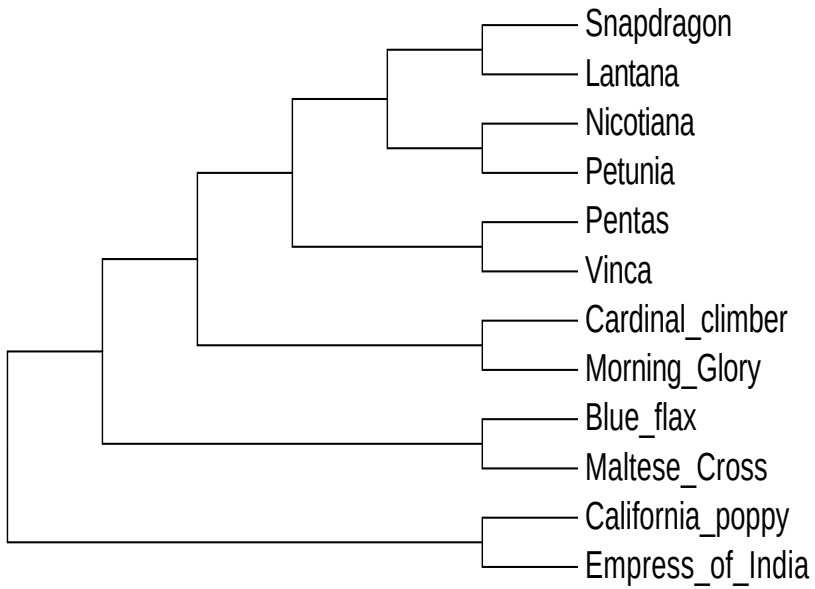
2. Vortex and centrifuge all reagents.
3. Label six 0.2mL PCR tubes: 1-4, +, and -. Include your group identifier on each as well.
4. Prepare your PCR master mix in a clean 1.5mL microcentrifuge tube. You will perform 6 PCR reactions (4 leaf extraction DNA samples, positive control, and negative control). To ensure you have enough PCR master mix, you will prepare for 7 reactions. Be sure to change your tip between each reagent.

1 PCR Reaction	7 PCR Reactions	✔
10µL REDExtract-N-Amp Ready Mix		
2µL of Forward Primer		
2µL of Reverse Primer		
2µL of Water		
4µL of Leaf Extract		
20µL Total Reaction		

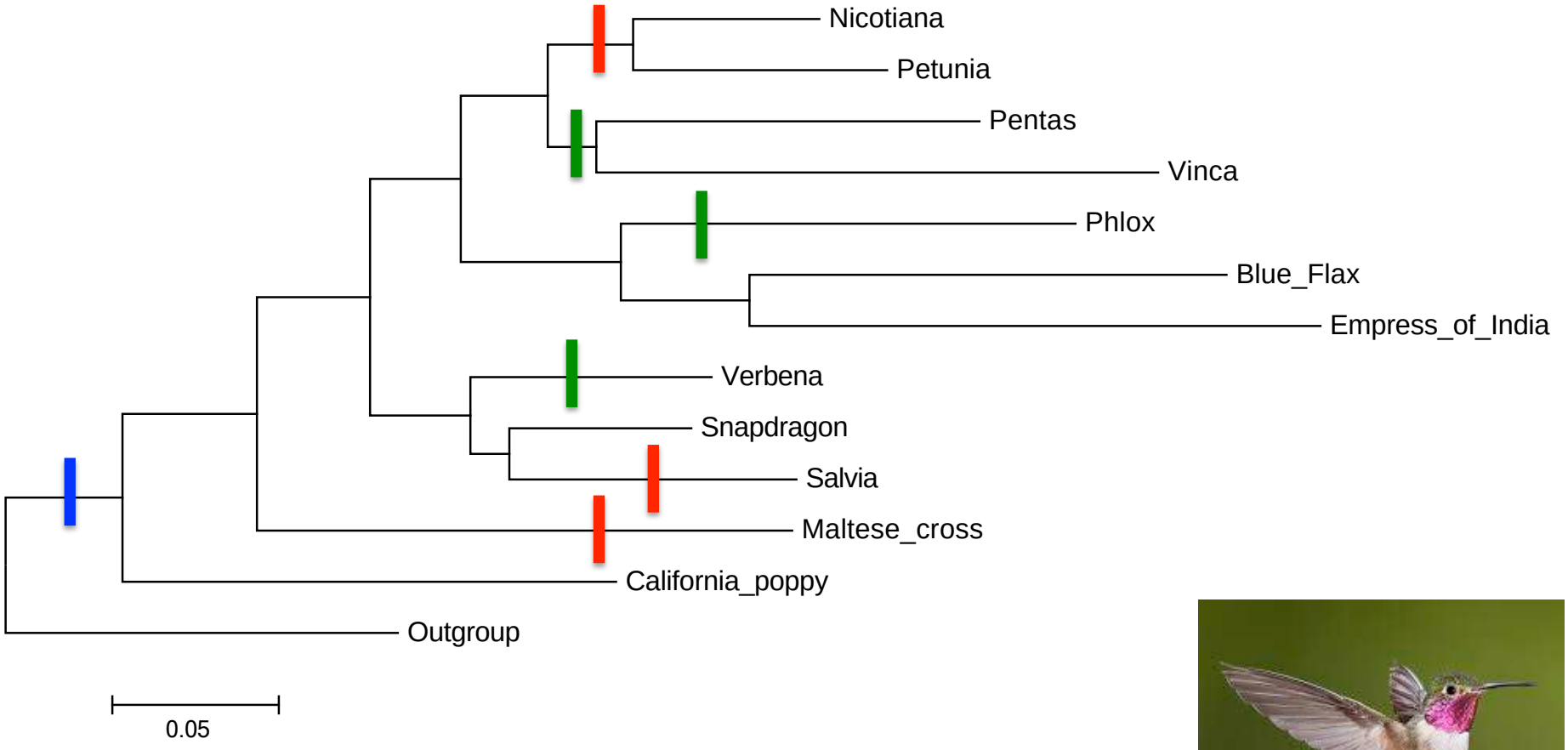
Molecular Phylogenetic Analysis

- Sequences downloaded from Genbank
  - ITS, most species have this available





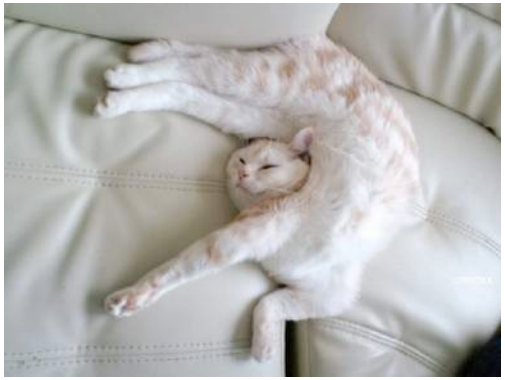
Character Mapping

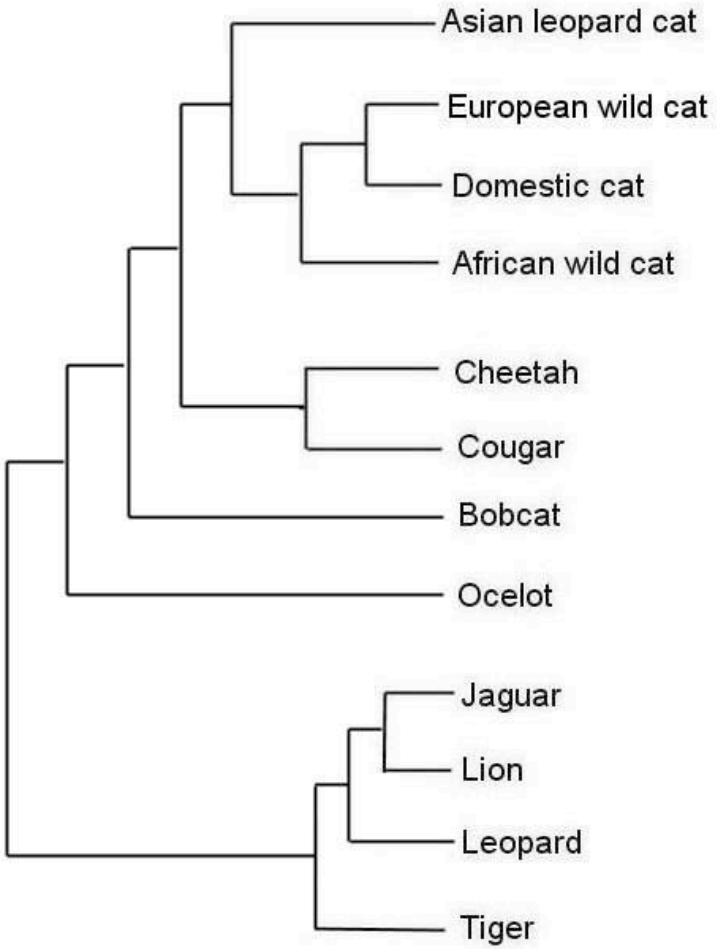


Bee Butterfly Hummingbird

Applications of module

Where did house cats come from?





(Redrawn after Johnson, et al, 2006)

NGSS Aligned

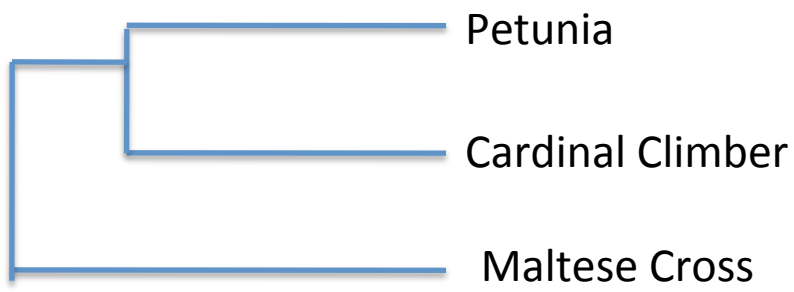
- Disciplinary Core Ideas
  - LS1.A: Structure and function; LS3.A: Inheritance of traits; LS3.B: Variation of traits; LS4.A: Evidence of common ancestry and diversity; LS4.B: Natural selection; LS4.C: Adaptation; can also segue into LS2: Ecosystems
- Nature of Science
  - Science models, laws, mechanisms, and theories explain natural phenomena; scientific knowledge assumes order and consistency in natural systems
- Practices of science
  - Analyzing and interpreting data; constructing explanations; engaging in argument from evidence; obtaining, evaluating, and communicating information; asking questions; developing and using models; science is a human endeavor
- Cross-cutting concepts
  - Patterns; cause and effect; systems and system models

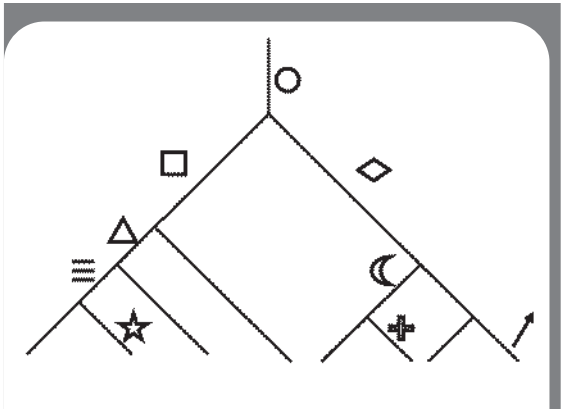
NGSS – Key Performance Expectations

- HS-LS3-1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS4-1. Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence. [Emphasis is on a conceptual understanding of the role each line of evidence has relating to common ancestry and biological evolution. Examples of evidence could include similarities in DNA sequences, anatomical structures, and order of appearance of structures in embryological development.]
- HS-LS4-2. Construct an explanation based on evidence that the process of evolution primarily results from four factors: (1) the potential for a species to increase in number, (2) the heritable genetic variation of individuals in a species due to mutation and sexual reproduction, (3) competition for limited resources, and (4) the proliferation of those organisms that are better able to survive and reproduce in the environment.
- HS-LS4-4. Construct an explanation based on evidence for how natural selection leads to adaptation of populations. [Emphasis is on using data to provide evidence for how specific biotic and abiotic differences in ecosystems (such as ranges of seasonal temperature, long-term climate change, acidity, light, geographic barriers, or evolution of other organisms) contribute to a change in gene frequency over time, leading to adaptation of populations.]
- HS-LS4-5. Evaluate the evidence supporting claims that changes in environmental conditions may result in: (1) increases in the number of individuals of some species, (2) the emergence of new species over time, and (3) the extinction of other species.

Modifications

- Students need increased scaffolding
  - The Great Clade Race (Goldsmith 2003)
  - Small trees first





- Flower species interchangeable
- Just morphological

In the Classroom

- Easy to break into 45 minute to hour segments (or longer)
  - Background information with floral features and pollinators
  - Picking and scoring flower features
  - Morphological analysis
  - DNA Extractions and/or PCR set up and running
  - Gel electrophoresis
  - Molecular analysis

Additional module

Forensic Botany in the High School Classroom: Real-World Application of Molecular Techniques

Materials

- http://www.cpet.ufl.edu/resources/plant-phylogenetics/
- http://people.clas.ufl.edu/jblandis/module-materials/

- Jacob Landis: jblandis@ufl.edu
- Julie Bokor: jbokor@ufl.edu