DNA Extraction

1. Rinse forceps and scissors with 70% ethanol prior to use and between handling different samples

2. Cut a roughly 0.5 cm wide piece of leaf material from a small, young leaf. Place into a 2 mL collection tube. Repeat to have a total of 4 extractions from different species.

3. Add 100 µL of Extraction Solution to the collect tube. Close tube and vortex briefly to mix. Make sure leaf material is covered by solution.

4. Incubate at 95°C for 10 minutes. Leaf tissue will not appear to be degraded.

5. Add 100 µL of Dilution Solution and vortex briefly to mix. Removal of leaf material is not necessary.

PCR

1. Remove REDExtract-N-Amp PCR Ready Mix, water, forward primer and reverse primer from fridge/freezer.

2. Each PCR reaction will contain the following quantities:

10 µL REDExtract-N-Amp Ready Mix
2 µL of Forward Primer
2 µL of Reverse Primer
2 µL of water
4 µL of leaf extract
20 µL total reaction

3. Calculate the amount of reagents needed to conduct PCR for the 4 extractions + a negative control + a positive control (total of 6 reactions).

4. Add appropriate reagents to PCR tubes.

5. Mix briefly and then centrifuge to collect all components at the bottom of the tube.

6. Place in thermocycler and run under the following conditions:

Initial denaturation at 95°C for 3 minutes
35 cycles of Denaturation at 95°C for 1 minute
Annealing at 52°C for 1 minute
Extension at 72°C for 1.5 minutes
Final extension at 72°C for 10 minutes
Hold at 4°C indefinitely

Prepare gel

1. Plug PowerBase™ into an electrical outlet.
2. Remove gel cassette from package
3. Insert the gel (with comb in place) into the base right edge first. The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the gel cassette in the PowerBase™. A steady, red light will illuminate if the gel cassette is correctly inserted.

Load prepared samples

Well #	1	2	3	4	5	6	7	8	9	10	11	12
What to add to the well	20µl dye mix	20µl dye mix	20µl dye mix	20µl dye mix	20µl dye mix	20µl dye mix	10µl water + 10µl PCR soln	10µl water + 10µl PCR soln	10µl water + 10µl PCR soln	10µl water + 10µl PCR soln	10µl water + 10µl PCR soln	10µl water + 10µl PCR soln

1. Remove and discard comb from the E-Gel® cassette.
2. In clean PCR tubes, combine 10 µl of water and 10 µl of PCR product.
3. In the first 6 wells, have students practice loading gel by adding 20 µl of loading dye mix to each well. Tubes from step 2 will be added in wells 7-12.

Run gel

1. Press and release the 30 minute button on the E-Gel® PowerBase™ to begin electrophoresis.
2. At the end of the run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping, and unplug the E-Gel® PowerBase™.
3. Remove the gel cassette and analyze your results by viewing on one of the transilluminators.

Flowers used in module

Hummingbird pollinated flowers

1) Annual Phlox (Red)
Phlox drummondii
family: Polemoniaceae

2) Cardinal climber
Ipomea quamoclit
family: Convululaceae

3) Petunia supercascade
Petunia x hybrida
family: Solanaceae

<u>Bee pollinated</u>
1) snapdragon, snappy bicolor
Antirrhinum majus
family: Plantaginaceae

2) Mealycup sage, Victoria
Salvia farinacea
family: Lamiaceae

3) Nasturtium, Dwarf, Empress of India
Tropaeolum majus

family: Tropaeolaceae

4) Blue Daze, dwarf morning glories

Evolvulus glomeratus

family: Convolvulaceae

<u>Butterfly pollinated</u>

1) Pentas

Pentas hybrida

family: Rubiaceae

2) Lantana

Lantana camara

family: Verbenaceae

3) Vinca

Catharanthus roseus

family: Apocynaceae

Unknown (to the students)

1) Annual Blue Flax (bee pollinated)

Linum usitatissimum

family: Linaceae

2) Nicotiana, Heaven Scent (hummingbird and hawkmoth pollinated)

Nicotiana alata

family: Solanaceae

Morphological phylogeny

- Have the students come up with 5 floral characters that they may use in order to group the plants in front of them by similarity. Some common ones will probably be flower color and flower size. See how creative they can be.

- Next have them complete the following table of characters by coding 0 (absence) or 1(presence) for characters. They will use this information to reconstruct a morphological phylogeny by hand.

species	Color (Red)	Color (Blue)	Flower size	Tubular flower	Symmertry	Petal lobes fused	Stamens exserted	Horizontal Orientation	Individual flowers	Scent	# of traits
Phlox	1	0	0	0	0	0	0	0	0	1	2
Cardinal climber	1	0	1	1	0	1	1	1	1	0	7
Petunia	1	0	1	1	0	1	0	1	1	1	7
Snapdragon	0	0	0	0	1	1	0	0	1	0	3
Salvia	0	0	1	1	1	1	1	1	0	0	7
Empress of India	0	0	1	0	1	0	0	1	1	0	5
Blue Daze	0	1	0	0	0	1	1	0	1	0	4
Pentas	0	0	0	1	0	0	0	0	0	0	1
Lantana	0	0	0	0	0	1	0	0	0	0	1
Vinca	0	0	1	0	0	0	0	0	1	1	3
Blue Flax	0	1	0	0	0	0	0	0	1	0	2
Nicotiana	0	0	1	1	0	0	0	1	1	0	4

Character 1
Red flower color
0 = not red
1 = red

Character 2
Blue flower color
0 = not blue
1 = blue

Character 3
Flower size
0 = small
1 = large

Character 4
Tubular flower
0 = not tubular
1 = tubular

Character 5
Symmetry of flower
0 = radial (like a wheel)
1 = bilateral (mirror images)

Character 6
Fused petal lobes
0 = not fused
1 = fused

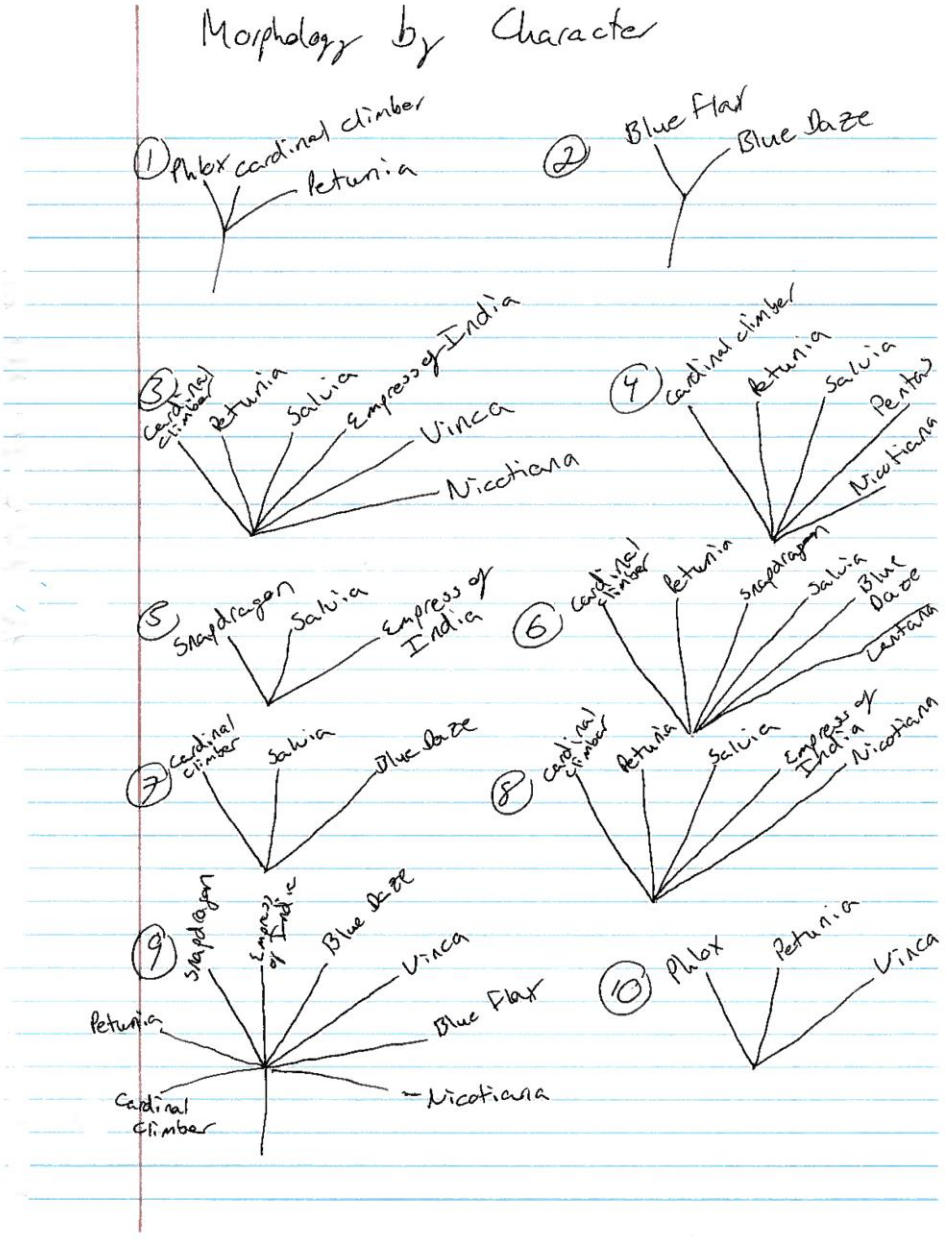
Character 7
Stamens exserted past petals
0 = not exserted
1 = exserted

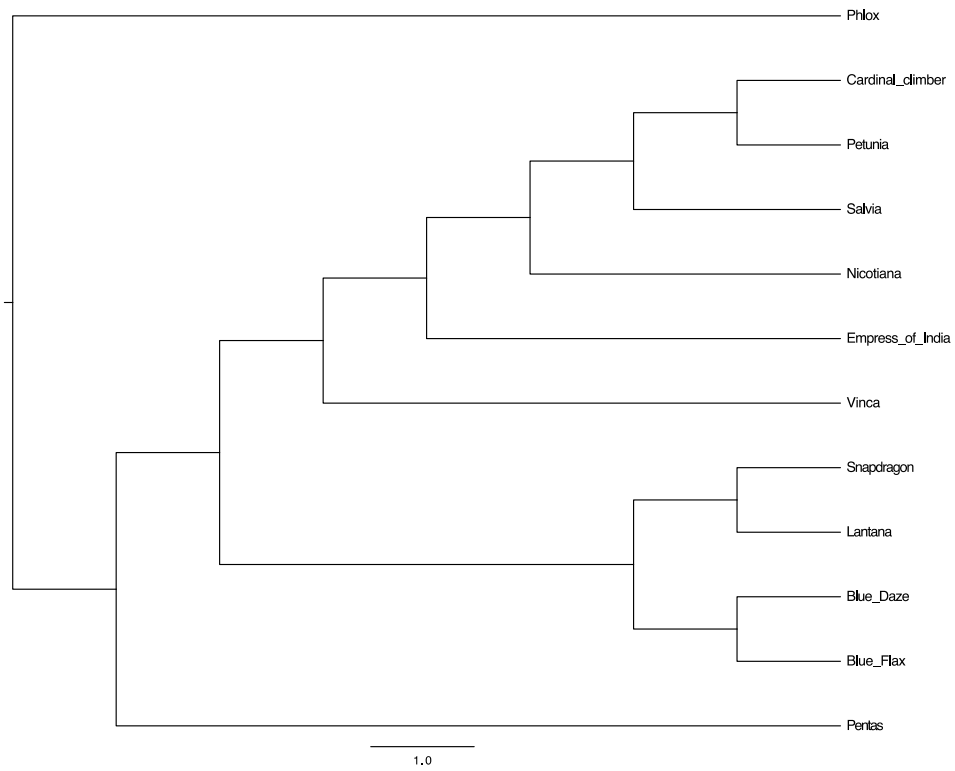
Character 8
Horizontal orientation
0 = not horizontal flower
1 = horizontal flower

Character 9
Individual flowers
0 = flower clusters
1 = individual flowers

Character 10
Scent
0 = no scent
1 = scent

When building the phylogeny by hand, I found it easiest to go through each character and draw which species it united together. There are 40 some potential trees possible with using these characters, so your over topology will likely be different. When I did this by hand, I found that character 4 was a good first step in getting things grouping together. Character 8 was my next informative character, followed by character 3 and then character 9. If you work on adding taxa for each of these groupings, you get a topology similar to what is found below. This also follows roughly with how many of the characters each species has from above. Cardinal climber, Petunia and Salvia all have 7 traits are imbedded deep in the tree. Where as Pentas (1) and Phlox (2) don't have many of the characters and are found towards the base of the tree.



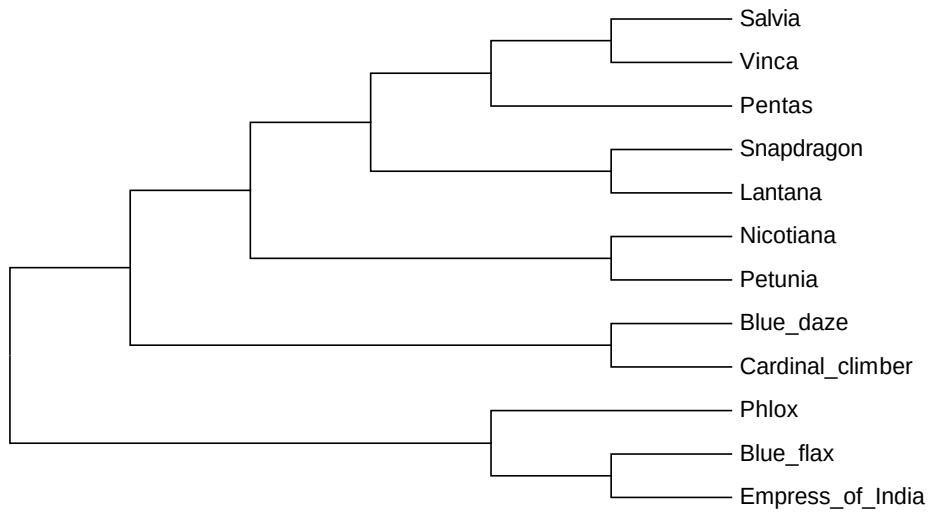


Morphological tree for all 10 characters

Molecular phylogenetic reconstruction

Use trnL-trnF sequences (12 total). We will walk through this part on the projector with everyone. Sequences are already loaded on the computers. Basically just start Mega, create a new alignment file, upload sequences, and do the clustal or muscle alignment. Using that file, we will create a very basic parsimony tree with default parameters.

Using Mega, we will do a muscle alignment on the sequences, and then run a parsimony analyses. Phylogeny looks like this:



State science standards, Next Generation Science Standards and associated module assessment questions

Plant Science

State Science Standards	Next Generation Science Standards	Module Assessment Question
SC.912.L.14.10 Discuss the relationship between the evolution of land plants and their anatomy. SC.912.L.14.53 Discuss basic classification and characteristics of plants. Identify bryophytes, pteridophytes, gymnosperms, and angiosperms. SC.912.L.14.7 Relate the structure of each of the major plant organs and tissues to physiological processes.	MS-LS1-4 Use argument based on empirical evidence and scientific reasoning to support an explanation for how characteristic animal behaviors and specialized plant structures affect the probability of successful reproduction of animals and plants respectively.	1) True or **False**: When scoring a particular floral trait, such as flower orientation, everyone scores that trait the same for each and every species. 2) Two species are most likely their closest relatives if: a) Their flowers are the same color and size **b) Phylogeny from molecular data** supports them being closely related c) They are found in the same geographic area d) All of the above

Evolutionary Theory

SC.912.L.15.1 Explain how the scientific theory of evolution is supported by the fossil record, comparative anatomy, comparative embryology, biogeography, molecular biology, and observed evolutionary change. SC.912.L.15.4 Describe how and why organisms are hierarchically classified and based on evolutionary relationships. SC.912.L.15.5 Explain the reasons for changes in how organisms are classified.	MS-LS4-2 Apply scientific ideas to construct an explanation for the anatomical similarities and differences among modern organisms and between modern and fossil organisms to infer evolutionary relationships. HS-LS4-1 Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.	3) You are part of a four-person research team performing a phylogenetic analysis with 100 species. Which method would you choose and why? Support your decision. a) 50 morphological characters (morphological phylogeny) **b) One gene consisting of 1,500 base pairs of DNA (molecular phylogeny)** - Possible answers: do not have to worry about everyone scoring characters the same, takes less time to gather data, results usually more robust than with morphology

DNA Technology		
State Science Standards	Next Generation Science Standards	Module Assessment Question
SC.912.L.16.11 Discuss the technologies associated with forensic medicine and DNA identification, including restriction fragment length polymorphism (RFLP) analysis. SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.	*NGSS does not focus on process skills. DNA technology is incorporated into the following standard and in relation to the Nature of Science.* MS-LS4-5 Gather and synthesize information about the technologies that have changed the way humans influence the inheritance of desired traits in organisms. Science is a Human Endeavor Technological: advances have influenced the progress of science and science has influenced advances in technology. (HS-LS3-3)	4) What is the correct order of techniques needed to determine relatedness of individuals using molecular data? a) PCR, DNA extraction, gel electrophoresis, phylogenetic analysis b) Phylogenetic analysis, gel electrophoresis, DNA extraction, PCR **c) DNA extraction, PCR, gel electrophoresis, phylogenetic analysis** d) Gel electrophoresis, DNA extraction, phylogenetic analysis, PCR 5) What is gel electrophoresis? a) Denaturing proteins into their gel state b) Identifying pieces of DNA by sequence c) Making Jello by using electricity to link the molecules **d) Passing electricity through a gel to separate molecules by size**