Supplemental Materials

Molecular Biology of the Cell

Hobbs *et al.*

Name: ____________________

Discussion Questions #1

At the start of Lab 3, you will discuss with your AI your reading of textbook pp. 7-15 (Plant Systematics, 2nd ed. Simpson 2010). Be prepared to answer the following questions:

*****Remember, you must bring typed answers to these questions to the discussion.*****

1. How would you define systematics in your own words?
2. What are the assumptions and primary goal of modern systematics?
3. How would you describe the four components of taxonomy in your own words and explain how they are related to each other?
4. What is an apomorphy? Give one example of an apomorphy from the family Lycopodiaceae.
5. In the discussion of phylogeny, the text repeatedly refers to common ancestors. Does this refer to an individual ancestral organism or to an ancestral species?

Exercise & in-class discussion #1 – Completing a character x taxon matrix

Think of 5 traits possessed by the three Lycopodiaceae genera we studied in Lab 2. List these in the "Characters" column in the table below and then indicate which form of this character is possessed by each genus. We did the first one for you to get you started.

Characters	*Huperzia*	*Lycopodium*	*Lycopodiella*
1. Gametophyte habit	subterranean	subterranean	surface-dwelling
2.			
3.			
4.			
5.			

****Include a copy of your completed table with your answers to the discussion questions above*****

Name: ____________________

Discussion Questions #2

At the start of Lab 4, you will discuss with your AI your reading of textbook pp. 17-24. Be prepared to answer the following questions:

*****You must bring typed answers to these questions to the discussion.*****

1. What is the difference between plesiomorphic and apomorphic conditions? Is heterospory considered a plesiomorphic or apomorphic condition for the Isoetopsida?
2. What is the difference between an ingroup and an outgroup? What is the immediate outgroup of the class Isoetopsida?
3. What is the relationship between characters and character states? If "protostele type" is a character, what would the character states be?
4. How are homology and homoplasy alike and how are they different? What is the difference between convergence and reversal?
5. Draw an unordered transformation series using the three types of protosteles. Next draw a hypothetical ordered series, and finally a hypothetical ordered and polarized transformation series for the protostele character states. In your own words, what is a transformation series?
6. What is a character x taxon matrix?

Exercise & in-class discussion #2 – Mapping character traits onto a tree

1. Finish filling in the character x taxon matrix below with characters and character states from the 5 genera we have studied in lab so far. We have chosen four characters from last week's exercise to get you started.

Characters	*Huperzia*	*Lycopodium*	*Lycopodiella*	*Selaginella*	*Isoetes*
1. Gametophyte habit	Subterranean exosporic	Subterranean exosporic	Surface dwelling exosporic	endosporic	endosporic
2. Sporophyll organization					
3. Type of stem branching					
4. Gemmae					
5.					
6.					
7.					

2. Draw the phylogenetic tree which shows the five genera we have studied in the division Lycopodiophyta (see the lecture notes from lecture 2). Now, map your character state changes from your matrix above onto this tree. Remember, you will have to make decisions regarding which character states you will consider ancestral.

****Include a copy of your completed character x taxon matrix and your tree with your answers to the discussion questions above*****

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Discussion Questions #3

At the start of Lab 6, you will discuss with your AI your reading of textbook pp. 24-40. Be prepared to answer the following questions:

*****Remember, you must bring typed answers to these questions to the discussion.*****

1. In the example of cladogram construction developed in Fig. 2.7, which characters are synapomorphies, which character is an autoapomorphy, and which character is homoplastic?
2. How does the interpretation of a cladogram change with the alternative presentations in Figs. 2.6E, 2.8A, 2.8B, and 2.8C?
3. The principle of parsimony is related to the concept of Ockham's Razor in which, all things otherwise equal, the simplest hypothesis is accepted as the preferred explanation. But is the simplest hypothesis necessarily correct? Provide an example to support your hypothesis.
4. What is the difference between Acctran and Deltran character optimization?
5. Although most phylogenetic analyses do not consider reticulation when constructing cladograms, does the most parsimonious dichotomously branched cladogram provide an accurate estimate of evolutionary relationships when reticulation does occur?
6. What is a strict consensus tree?
7. What is the consistency index (CI)?

Exercise #3 – Creating a phylogenetic tree

	Outgroup			**← Ingroup →**		
Characters	***Lycopodiacae***	***Ophiogloss-aceae***	***Psilotaceae***	***Equiset-aceae***	***Polypodi-aceae***	***Marsileaceae***
1. Spore condition	homosporous			homosporous		heterosporous
2. Gameto-phyte habit	subterranean	subterranean				surface dwelling
3. Stele	protostele			siphonostele		dictyostele
4. Leaf	microphyll		microphyll*		macrophyll	
5. Sperm	biflagellate			multiflagellate		

* Some species in Psilotaceae, such as *Psilotum nudum* which you saw in lab, have enations instead of microphylls, but for the purpose of this exercise we will use microphyll as the character state for this family.

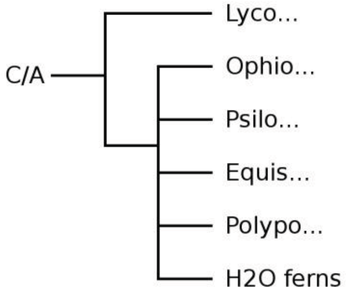
Above is a character x taxon matrix of morphological characters for several taxa in Polypodiophyta. Use the following process (partially described on pages 24-25 in your textbook) to construct a phylogenetic tree using the fewest possible character state changes.

a. Complete the character x taxon matrix above by filling in the missing character states (this is good review for the upcoming lecture exam!) How can you tell if these character states are derived or ancestral?

b. Draw a polarized, ordered transformation series diagram for each character. Polarize each character's character states based on your knowledge of which states are ancestral. As an example we did the character "Stele" for you below.

(Ancestral state) → (1st derived state) → (2nd derived state)
Protostele → Siphonostele → Dictyostele

c. Begin creating your tree by going through the process on page 25 in your text book. On a piece of scratch paper, draw a tree which has a polytomy containing all of the ingroup taxa, and which has the out group attaching below the polytomy. See the example below and textbook figure 2.6-C (page 25)

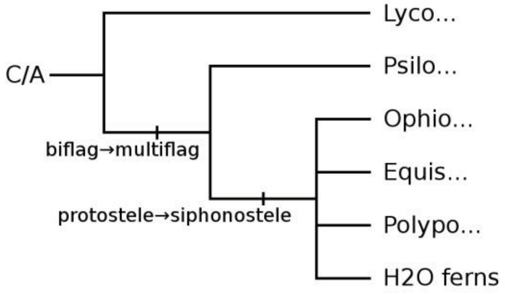


Are there any derived character states shared by all of the members of the ingroup? Remembering the principle of parsimony, add the character state change/s required for this situation to the proper location on your tree. Which character state change/s did you add?

Now, find the next most commonly shared derived character state in the matrix.

Note: taxa with derived character states in the 2nd position in a series (such as "dictyostele") had ancestors who once possessed the derived character state in the 1st position. This means that "siphonostele" is the next most common derived character state because four taxa either currently or once shared this trait. Do you need to modify your tree to add this derived character state? If so, redraw your tree, grouping together taxa which share this apomorphy to separate them from the other taxa.

At this point your tree should look like this:



Now, find the next most commonly shared derived character state in the matrix. If there is more than one, pick one and go with it. Remember to redraw your tree if necessary by grouping together taxa which share the apomorphy to separate them from the other taxa.

Continue this process until all of the character states in the matrix are on your tree. Remember to do this on scratch paper, redrawing your tree as necessary.

d. Your finished tree will look different than those presented in lecture for Polypodiophyta. Why would this be?

e. Are there any instances of homoplasy in your tree? Where? Does it involve reversal or convergence?

f. What is the minimum number of character state changes that could occur? How many character state changes actually do occur in your tree? Calculate your tree's CI index. CI = (min number)/(actual number)

For this exercise please turn in your answers to the questions above & a copy of your final tree.

Name: ____________________

Exercise #4 – Creating a phylogenetic tree

	Outgroup	**← Ingroup →**					
Characters	***Lycopodiacae***	***Psilotaceae***	***Ophioglossaceae***	***Equisetaceae***	***Marattiaceae***	***Polypodiaceae***	***Marsileaceae***
1. Spore condition	homosporous	homosporous	homosporous	homosporous		homosporous	heterosporous
2. Gametophyte habit	subterranean	subterranean	subterranean	surface dwelling		surface dwelling	surface dwelling
3. Sporangia type					eusporangia		leptosporangia
4. Stele	protostele	protostele	siphonostele	siphonostele	dictyostele	dictyostele	dictyostele
5. Leaf	micro	micro*	macro	micro		macro	macro
6. Sperm	biflagellate	multi	multi	multi		multi	multi

* Some species in Psilotaceae, such as *Psilotum nudum* which you saw in lab 4, have enations instead of microphylls, but for the purpose of this exercise we will use microphyll as the character state for this family.

Above is a character x taxon matrix of morphological characters for several taxa in Polypodiophyta. Use the following instructions to construct a phylogenetic tree of this data using the fewest possible character state changes. This method is somewhat different from the one you used last week and will be used again later in the course.

a. Complete the character x taxon matrix above by filling in the missing character states for the character "sporangia type" and the taxa "Marattiales"

b. Complete the table below to polarize the character states. Since Lycopodiaceae is the outgroup, we will consider its traits to be ancestral. Assign each character state a "0" if it is ancestral, a "1" if it is derived 1 step from the ancestral state, and a "2" if it is derived 2 steps from the ancestral state. We did the first one for you to get you started:

Spore condition: Heterosporous = 1 Homosporous = 0
Sporangia type: Leptosporangia = Eusporangia =
Gametophyte habit: Subterranean = Surface dwelling =
Stele: Protostele = Siphonostele= Dictyostele=
Leaf: Macrophyll = Microphyll =
Sperm: Biflagellate = Multiflagellate=

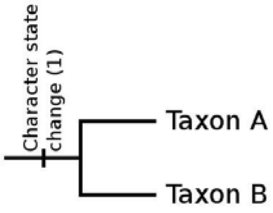
c. Now, recode the character x taxon matrix using 0s, 1s, or 2s. To do this, fill in the table below with 0s, 1s, or 2s using the information from step (b). To get you started we did the row "Stele" for you.

	Outgroup	**← Ingroup →**					
Characters	***Lycopodi-acae***	***Psilot-aceae***	***Ophiogloss-aceae***	***Equiset-aceae***	***Maratti-aceae***	***Polypodi-aceae***	***Marsile-aceae***
1. Spore condition							
2. Gametophyte habit							
3. Sporangia type							
4. Stele	0	0	1	1	2	2	2
5. Leaf							
6. Sperm							
Total # of character state changes for taxa							7

d. In the last row of the table above, enter the total number of character state changes that occurred in the lineage of each taxa. We did Marsileaceae for you.

e. Now, within each row, circle groups of derived characters which are next to each other. For example, the three "2s" in the row "Stele" are circled because they are the same number and are next to each other. This shows that the Marattiales through Marsilaceae share a common ancestor who had a dictyostele. This group of "2s" is included as part of a larger group which includes two taxa with "1s". This indicates that Ophioglossaceae through Marsilaceae share a common ancestor who had an ectophloic siphonostele. So, we see that this process groups together taxa in order to show which taxa share a character trait through common ancestry.

f. Now you're ready to start drawing the actual phylogenetic tree. Look for any circles which enclose a group of two taxa and connect them together on piece of scratch paper like this:



Next, look for groups of three and add that group to your tree. If some of the taxa of the next group are already on the tree, don't add them again, but simply add any new taxa to the tree you've already drawn. Continue this process until the tree is finished. Don't forget to include the character state changes!

g. Your finished tree should look different than the one presented in the lecture notes for Polypodiophyta. Why is this? Verbally describe the differences and similarities between these two trees.

h. Are there any instances of homoplasy in your tree? Where? Is this an instance of reversal or convergence?

i. What is the minimum number of character state changes that could occur? How many character state changes actually do occur in your tree? Calculate your tree's CI index. CI = (min number)/(actual number)

For this exercise please turn in your answers to the questions above & a copy of your final tree.

Name: ____________________

Exercise #5 – Creating a phylogenetic tree from DNA data

*****Please complete this exercise and bring your answers with you to next week's lab*****

Presented below is a set of taxa with their corresponding DNA sequences, all of which code for the same mitochondrial gene product. (Note: the sequences for this exercise are made up, though the outgroup sequence is actually a portion of the chloroplast DNA sequence in *Polypodium hesperium*, the western rockcap fern) The nucleotide positions are identical in concept to the different morphological characters we have used in previous exercises. In fact, we could have labelled each column in matrix as character 1, 2, 3, etc.

	Nucleotide Position:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	Polypodium:	C	A	T	T	G	C	A	A	G	G	G	G	A	C	T	G	A	A	G	C	C	T	G	C	T
↑ Ingroup ↓	*Cycas*:	C	A	T	T	G	G	A	G	C	G	C	G	A	C	C	G	A	C	G	C	C	T	C	C	T
	Zamia:	C	A	T	T	G	G	A	G	C	G	C	G	A	C	T	G	A	C	G	C	C	T	C	C	T
	Ginkgo:	C	A	A	T	G	G	A	A	G	A	G	G	A	C	T	G	A	C	G	C	C	T	C	C	T
	Araucaria:	C	A	A	T	G	G	A	A	G	G	G	T	A	C	G	G	A	C	G	C	C	T	C	C	A
	Taxus:	C	A	A	T	G	C	A	A	G	G	G	T	A	C	G	G	A	C	G	C	C	T	C	A	A
	Podocarpus:	C	A	A	T	G	C	A	A	G	G	G	T	A	C	G	G	A	C	G	C	C	T	C	A	A
	Cupressus:	C	A	A	T	G	C	A	A	G	G	G	T	A	C	G	G	A	C	G	C	C	T	C	A	A
	Pinus:	C	A	A	T	G	G	A	A	G	G	G	T	A	A	G	G	A	C	G	C	C	T	C	C	T
	Ephedra:	C	A	A	T	T	G	A	A	G	G	G	C	A	A	G	G	A	C	G	C	C	T	A	C	T
	Gnetum:	C	A	A	T	T	G	A	A	G	T	G	T	A	A	G	G	A	C	G	C	C	T	A	C	T
	Welwitschia:	C	A	A	T	T	G	A	A	G	T	G	T	A	A	G	G	A	C	G	C	C	T	A	C	T
	Magnolia:	C	A	T	T	G	C	A	A	C	G	G	C	A	C	A	G	A	A	C	C	C	T	C	G	T

Linking taxa:

Using *Polypodium* as your outgroup, link together taxa which share synapomorphies by circling derived character states which are next to each other. For example, the nucleotide G is the ancestral trait for position 14. Most of the ingroup taxa (*Cycas* through *Magnolia*) have the ancestral condition, but the nucleotide T is present in four taxa (*Pinus* through *Welwitschia*). Circle these four Ts. This synapomorphy was likely the result of a mutation (from G to T) that was inherited from the common ancestral lineage of these four taxa. Also circle any autapomorphies (a derived nucleotide found only in a single taxa) that are present.

Transformation series:

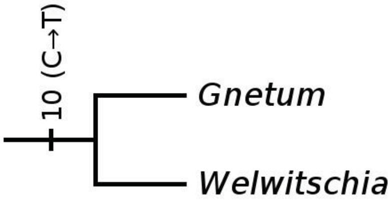
With DNA sequences each nucleotide may potentially mutate to any other nucleotide. For example, the ancestral state at position 12 is T, and there are two derived states, G and A. Either of these could have arisen directly from T, or could be the second step of a three-step transformation series. This means that it is not possible to make transformation series prior to actually constructing the phylogenetic tree. Fortunately, you don't need to know this information to construct the tree, as following the principles of parsimony will be sufficient. The transformation series for these character traits will emerge as you construct the tree according to the principles of parsimony.

Uninformative characters:

As is frequently the case in DNA data, you will notice that many of the positions in the sequence do not have any mutations (for example, position 1 and 2). These nucleotide positions are considered uninformative and can be ignored as you construct your tree.

Constructing the phylogenetic tree:

Once you have finished circling groups of derived character states you're ready to start drawing the actual phylogenetic tree. Look for a circle which encloses a group of two taxa and connect these taxa together to show that they had a common ancestor. For example, at position 10 we see that *Gnetum* and *Welwitschia* share the derived nucleotide T. We can connect these taxa together as follows:



Look for other groups of two and add them to your tree. If the taxa are already on your tree simply add the character state change to the existing lineage. Next, look for groups of three, then four, etc. Last, add any autapomorphies.

Questions:

1. When considering a DNA data set for phylogenetic analysis, does the sequence position of base pairs correspond to the morphological characters we have used in past exercises? If sequence positions are a type of character, what is equivalent of character traits in a DNA data set?

2. Are there instances of homoplasy in your tree? Where? In each case, is this an instance of reversal or convergence?

3. What is the minimum number of nucleotide mutations that must occur? Calculate this by determining how many different derived nucleotides there are in each column of the matrix (write this number at the base of each column), and then add all of these column subtotals to obtain a single value for the matrix.

4. How many nucleotide changes actually occur in your tree? Calculate the Consistency Index (CI) of your tree.

5. Draw an ordered and polarized transformation series for nucleotide position 12.

6. Can you find any traits which could be mapped using either Acctran or Deltran approaches?

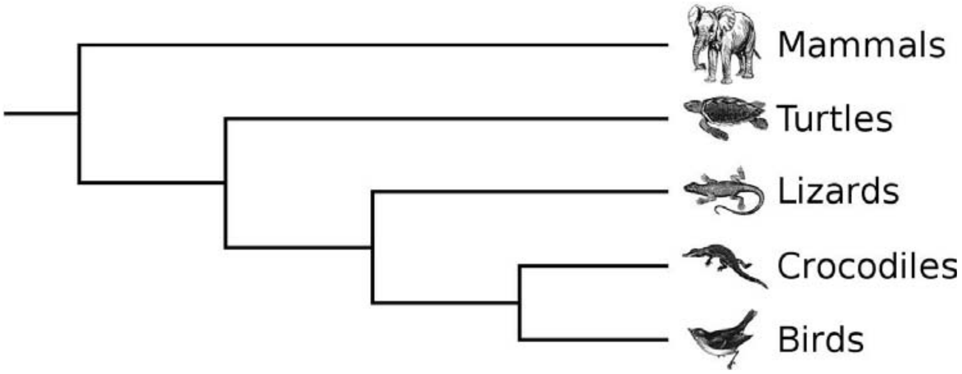
****For this exercise please turn in your answers to the questions above & a copy of your final tree.****

Name: ____________________

Phylogenetic analysis concept inventory

This survey is designed to gauge your understanding of phylogenetic trees. It is intended to aid in the development of better instructional materials and practices. Please answer the questions as best as you can.

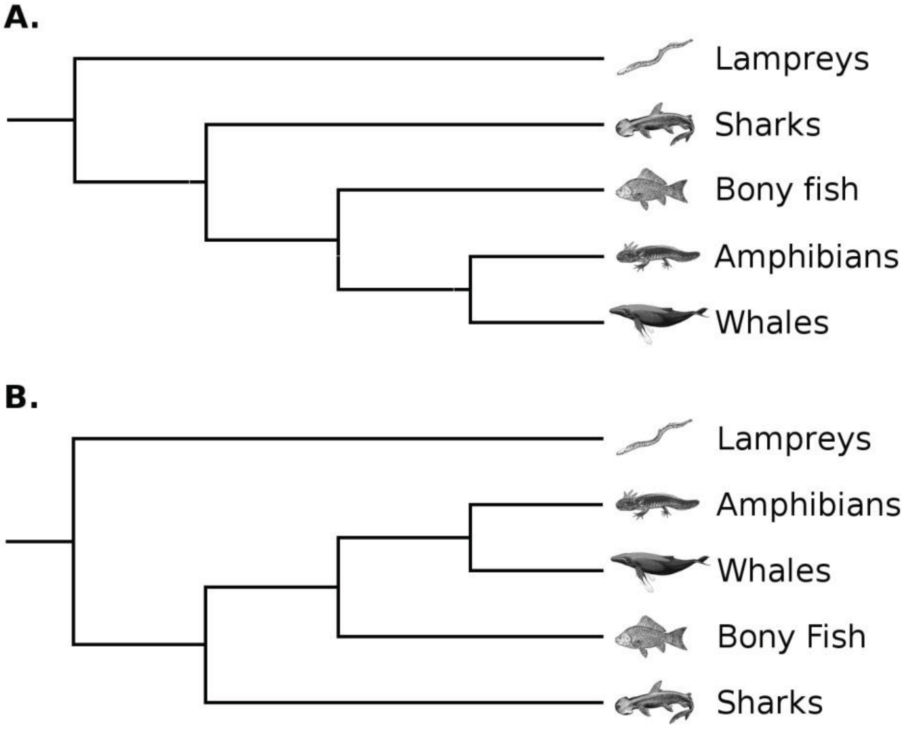
1. Which of the five taxa shown on the phylogenetic tree below would you consider to be the most evolved? Why?



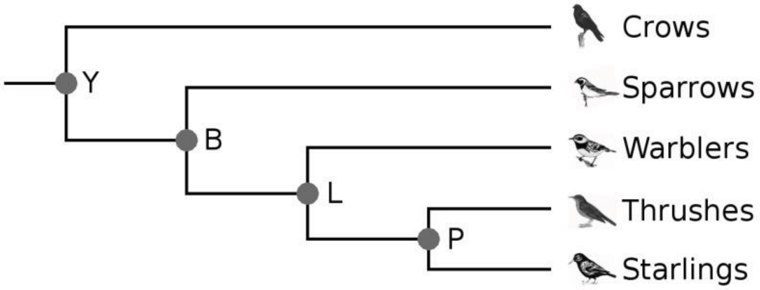
2. Draw an arrow on the above diagram which shows the direction of time from past to present.

3. Explain why you think the following statement is true or false: Organisms which share traits are closely related and should occur next to each other on a phylogenetic tree.

4. Explain whether the two trees below show the same or different phylogenetic relationships among the taxa.



5. In the phylogenetic tree shown below are sparrows more closely related to crows or starlings? Common ancestors are shown as lettered circles at the nodes (Y, B, L, and P). How did you come to your conclusion?



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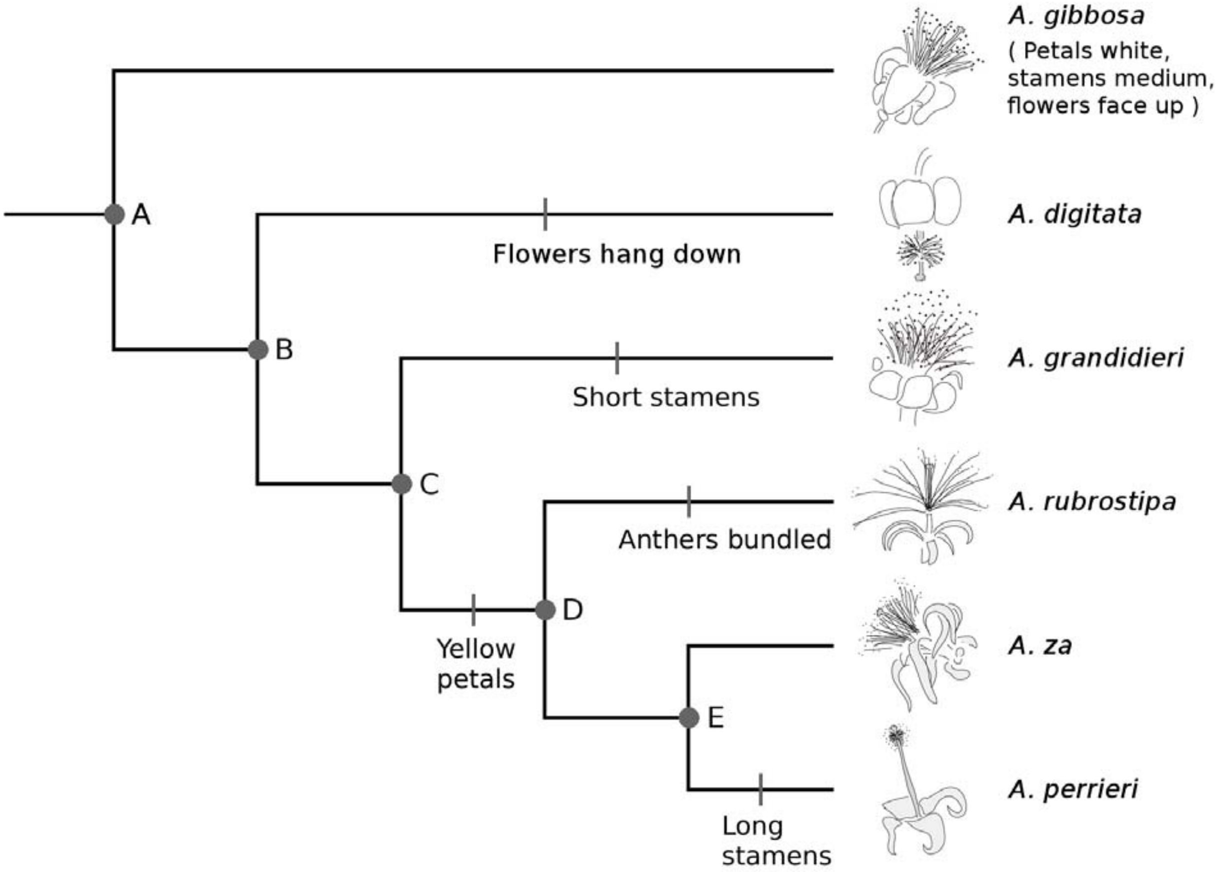
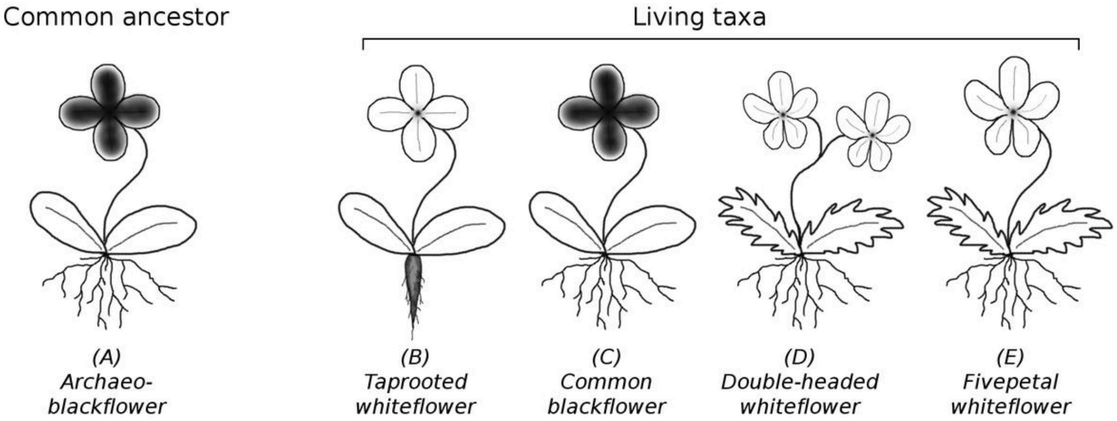
6. The diagram below shows the possible evolutionary relationships among six living species of baobab trees. The drawings show the flowers of the living baobab species. The letters A- E represent ancestral species.



For each of the following three traits, circle the option which describes what the flowers of species D would have looked like according to this phylogenetic tree:

Petals: a. White b. Yellow c. Can’t tell from diagram
Stamens: a. Short b. Medium c. Long d. Can’t tell from diagram
Flowers: a. Face up b. Hang down c. Can’t tell from diagram

7. The figure for this question shows four present-day (living) taxa and their extinct common ancestor. The living taxa differ from each other in five ways: leaf shape, root type, flower color, number of flowers, and number of petals. Construct a phylogenetic tree which shows the evolutionary history of these taxa based on their traits.



Finally, in the space below, walk us through the thought process you used to create your phylogenetic tree above. A few sentences would be fine.

Coding rubric for the phylogenetic pre-/post-concept survey – Version A

The answers below are ordered according to **Version A** of the pre-post survey.

1. Which are most evolved? Why?:
    a. (3)Unsure: The organism which has evolved (changed) the most from the common ancestor is the one with the greatest number of character state changes and this tree doesn't contain that information
    b. (2) Birds and crocodiles OR (1) Birds: because they have more common ancestors than the other organisms (implicitly assuming that each node involves evolutionary change)
    c. (2) All are equally evolved (derived): because they are all alive today. (confusing extant and evolved)
    d. (1) Birds: because they are at the bottom of the tree
    e. (1) Mammals: because located at the top of the tree
    f. (0) Student used reasoning not related to interpreting the tree (ex: Mammals are smart so therefore are highly evolved)
    g. (0)No answer

2. Time:
    a. (1) Arrow from left (past) to right (present): correct
    b. (0) Arrow from top (past) to bottom (present): incorrect
    c. (0) Arrow from bottom (past) to top (present): incorrect
    d. (0) Arrow from right (past) to left (present): incorrect
    e. (0) No arrow: incorrect

3. True/False statement on homoplasy and relationship between common traits and common ancestry:
    a. (3) False/May be true or false: Common traits and common ancestry aren't necessarily linked. Includes 'homoplasy', 'convergence', 'reversal', or a synonym of these in their answer.
    b. (2) False/May be true or false: Common traits and common ancestry aren't necessarily linked (implied).
    c. (1) False: Position on tree is due to evolutionary descent, common traits don't matter (ignores that evolutionary descent is largely determined by common traits)
    d. (1) True: 'Relatedness' is determined by shared traits, so two organisms which share a trait should be closely related. (problem here: ignores homoplasy)
    e. (1) True: Shared traits are a result of common ancestry, so they should be close (also ignores homoplasy)
    f. (1) False with no rationale
    g. (0) No answer / True with no rationale

4. Ability of trees to rotate on nodes:
    a. (1) Correct: Relationship does not change with rotation
    b. (0) Incorrect: Relationship changes with rotation

5. Relatedness depends on common ancestry (node counting misconception):
    a. (3) Unsure: Relatedness is ultimately dependent upon the number of character state changes between organisms. Sparrows could be more related to crows or starlings depending on this.
    b. (2) Sparrows more closely related to starlings because they share a common ancestor at point "B" and so share common traits that sparrows don't share with crows. Or: sparrows & starlings have a more recent common ancestor.
    c. (2) All equally related: confusion with being extant & relatedness
    d. (1) Sparrows are more closely related with crows because there are fewer nodes between sparrows and crows than between sparrows and starlings
    e. (0) No answer

6. Skill test: tree reading / Deducing ancestral traits. Score from 0 – 3 based on how many questions are answered correctly

    Assign score of 1 (correct) or 0 (incorrect) for each question

    Question 1: correct answer = b
    Question 2: correct answer = b
    Question 3: correct answer = a

7. Skill test: tree construction. Score from 0-4 based on the following:

    Component 1: Branching pattern (is the topology correct?):
        (1) The three nodes in the tree show the correct relationships
        (0) One or more of the three nodes is in error

    Component 2: Placement of the extinct common ancestor:
        (1) Correctly placed at the base of the tree
        (0) Incorrectly placed as an extant outgroup or otherwise or not shown

    Component 3: Mapping character state changes:
        Version A has a tree with 5 character state changes, so assign (0.2) points for each correctly mapped character state change.

    Component 4: Alignment of living taxa at the end of the tree to show they are extant
        (1) Taxa are all aligned vertically at the end of the tree
        (0) Taxa are not aligned, are placed in-line with tree, or are not provided