

Molecular Phylogeny

Purpose

- to show how data about molecules can be used to find evolutionary relationships.

Introduction

Since all living things descended from a common ancestor, their cellular components (DNA, RNA, protein, etc.) share a common origin. Originally, there was only one species of life on earth. However, mutations occurred in its DNA, resulting in the production of different proteins in different individuals of that organism and their descendants. Once some of these descendants became different enough to be reproductively isolated from the parent a new species was formed. The resulting two species are then subject to further mutation and evolution.

In this lab, we will use the amino acid sequence of the protein cytochrome c as a 'molecular clock'. Cytochrome c is an essential part of cellular respiration and was presumably present in the first air-breathing ancestor of all modern animals and plants. As a result of this, all modern air-breathing plants and animals have cytochrome c's that are evolutionary descendants of the original cytochrome c. Since much time has passed since the ancestor existed, there have been many mutations in the cytochrome c gene and thus many changes in the amino acid sequence of cytochrome c.

Two organisms of the same species should have identical cytochrome c molecules. The longer the time since two organisms had a common ancestor, the more different the cytochrome c molecules will be. We will compare the amino acid sequences of cytochrome c from various organisms to determine their degree of evolutionary relatedness.

There are two main methods for comparing protein sequences from different organisms in order to determine their phylogenetic relationships:

- **Sequence Divergence** This compares the sequences and counts the number of differences between them. The longer since their common ancestor, the more differences expected. This is the simplest method. You will do this 'by hand' to see how it works and then let the computer do the hard work. This method is best for finding approximately how long it has been since two species had a common ancestor. It works fairly well for finding out which creatures are related to which. In studies of cytochrome c from many organisms, it has been found that (very approximately) one amino acid change occurs every 21 million years. The rates of change of other proteins are different.
- **Parsimony** This is a more sophisticated method that also takes into account the particular differences between the sequences. It is described in detail in *Campbell* pages 542-547. Although it can not tell you how long ago two organisms had a common ancestor, it is much better at telling which creatures are most closely related to which than the Sequence Divergence method.

In this lab, you will use both methods to see their strengths and weaknesses. You should remember that the software generates the most likely tree, but not necessarily the way the organisms actually evolved.

You will need your copy of *Campbell* for this lab.

Phylogenetic Trees

For the purposes of some pre-labs, etc., you will be asked to draw a partial phylogenetic tree showing the relationships between various organisms. Here is a hypothetical example to show you what we are looking for.

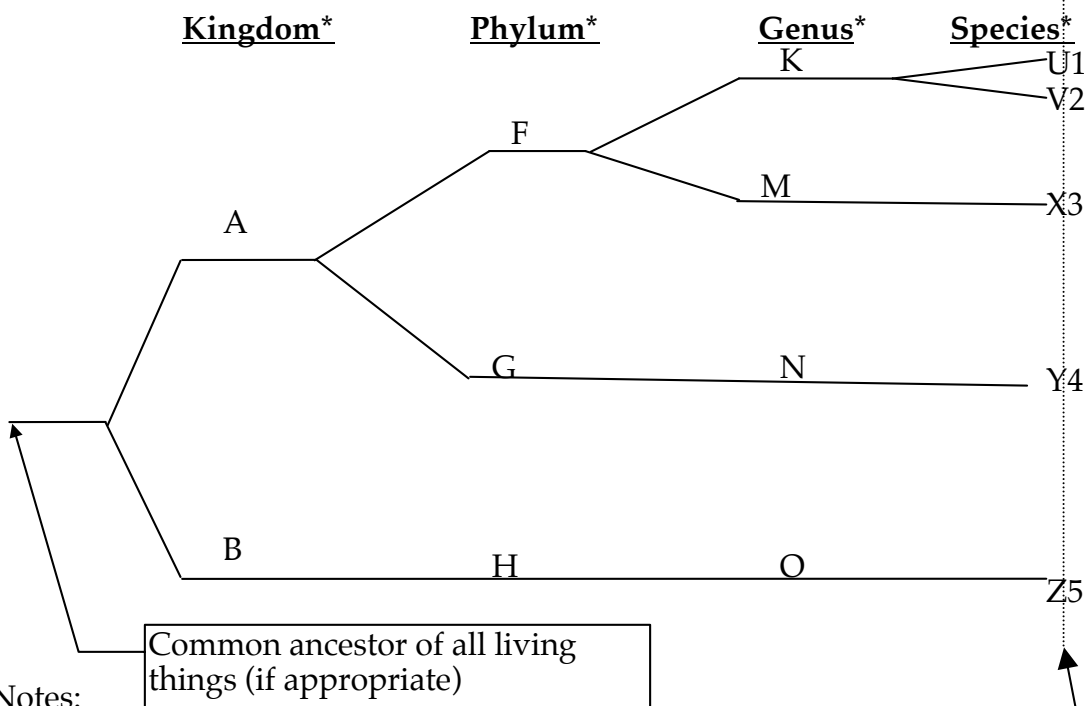
Given organisms 1 through 5 with the following classifications:

<u>Organism</u>	<u>Kingdom</u>	<u>Phylum</u>	<u>Genus</u>	<u>Species</u>
1	A	F	K	U
2	A	F	K	V
3	A	F	M	X
4	A	G	N	Y
5	B	H	O	Z

Thus:

- the difference between 1 and 2 is at the species level - they are different.
- the difference between 2 and 3 is at the genus level - they are in the same kingdom and phylum, but still are rather different.
- the difference between 3 and 4 is at the phylum level - they are in the same kingdom but still very different.
- the difference between 1 and 5 is at the kingdom level - they are extremely different.

This is shown in the diagram below: (this is what we will want on pre-labs, etc.)



Notes:

- since all species listed are currently alive, they must line up vertically, like this:
- the only distances that matter are the horizontal ones; vertical positions don't matter
- since all the species listed are currently alive, the distance from any one to the common ancestor must be the same.

* If given.

Procedure

You will work in groups of three per computer in this lab. The instructions in the manual are for the Macintosh computers; you can also access all of the resources for this lab from any computer with www access – no special plug-ins are required.

Part I: Sequence Divergence “The hard way” (you do half of the work)

In this part, you will use the software to show you the number of differences between two protein sequences - this will help you to understand how this information is generated. You will then use this information to construct a simple tree manually.

- 1) To access the “Tree Constructor”, start Safari from the Dock.
- 2) Click on the link to the OLLM and then the link for the “New Phylogenetic Tree Constructor”.
- 3) You will see a page that looks like this:

Main Tree Organisms
Select several organisms here to construct the tree.

Animals	Plants	Other
ailanthus silkmoth American alligator Arabian camel brown garden snail bullfrog California gray whale carp chicken chimpanzee cow	buckwheat castor bean cauliflower China jute corn ginkgo hemp leek mung bean nasturtium	green alga

Select the creatures to include in your tree by clicking on their names in these lists. You can select as many as you want. To select more than one non-adjacent creature in the same list, hold down the apple key. (Click this button to clear & start over)

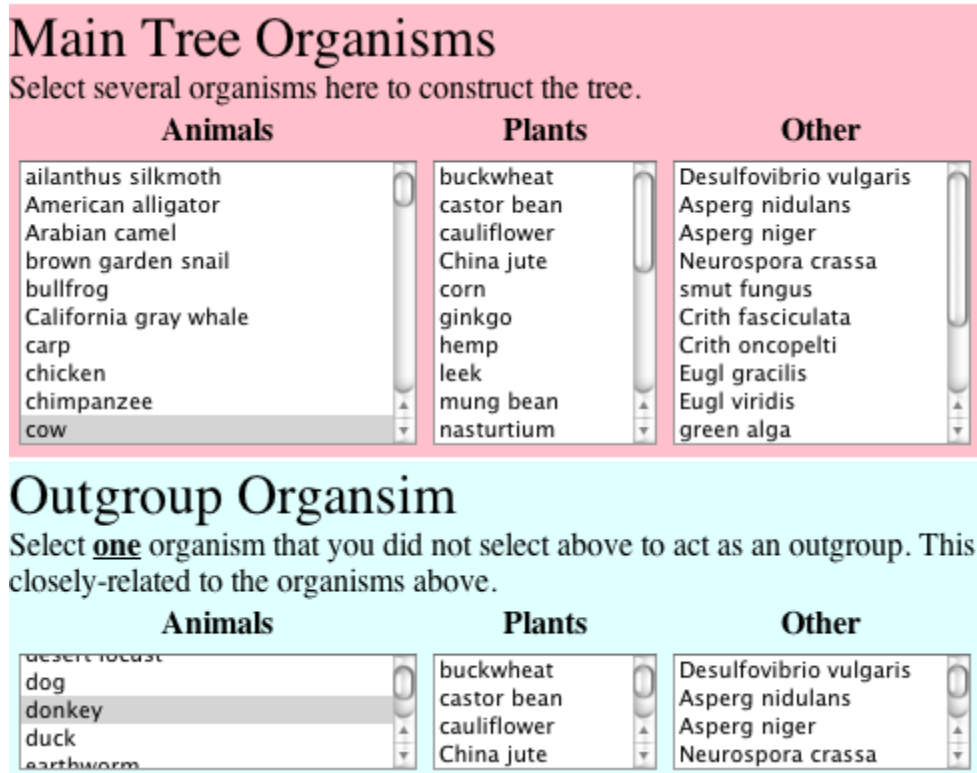
Outgroup Organism
Select one organism that you did not select above to act as an outgroup. This should be closely-related to the organisms above.

Animals	Plants	Other
bullfrog California gray whale carp chicken	buckwheat castor bean cauliflower China jute	Desulfovibrio vulgaris Aspergillus nidulans Aspergillus niger Neurospora crassa

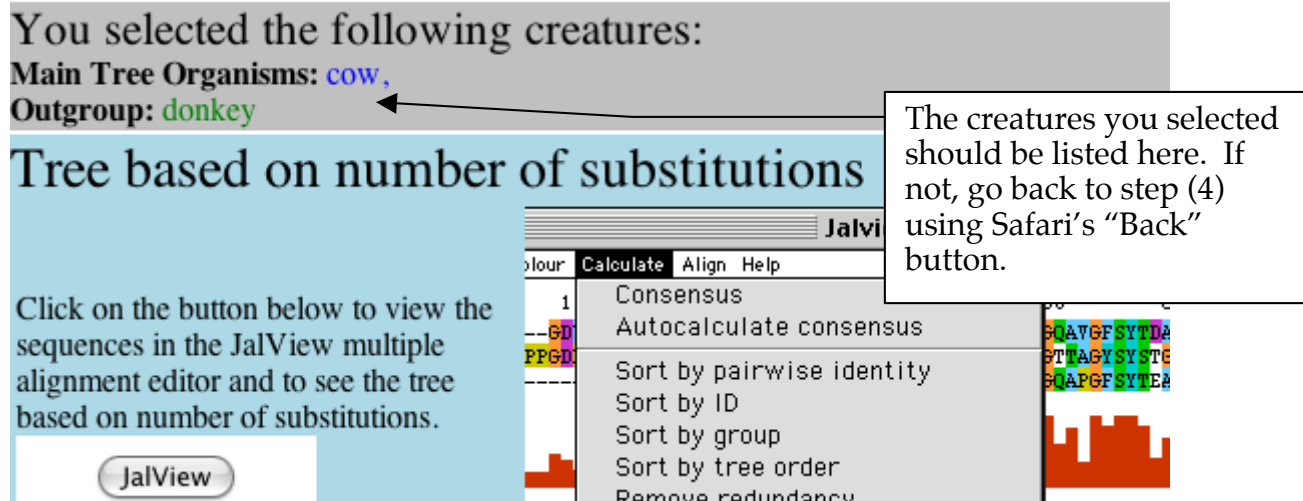
Calculate Tree Clear all selections

Click here when you are satisfied with your selections. it will submit your creatures to the server computer which will do the calculations.
These calculations may take a few minutes; please be patient.

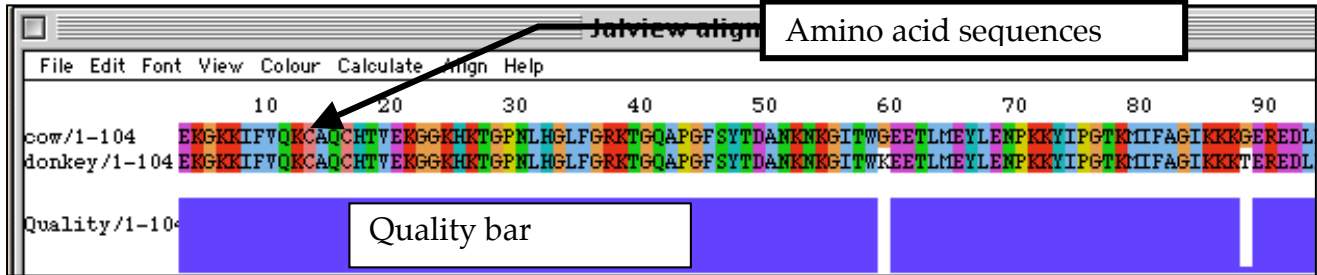
4) For this first exercise, we will use the program in a slightly unusual way. Choose two organisms that you think are closely-related. Select one in the “Main Tree Organisms” and one in “Outgroup Organism”. You have to select one in each set or the program will complain. In this example, I have chosen “cow” and “donkey”. You should choose two other organisms that are closely-related. The screen should look something like this (except your organisms are selected):



5) Click “Calculate Tree” and wait a little while and you should see this:



6) Click the “JalView” button and wait 20-60 seconds and you should see this (you may have to wait a little for all the colors to show):



This shows the amino acid sequence of cytochrome c from the cow (top line) aligned with the amino acid sequence of cytochrome c from the donkey (bottom line). There are several important features of this display:

- The amino acid sequences are listed left to right from amino to carboxyl ends.
- The length of the protein sequences is listed at the left end of the colored bands: “cow/1-104” means that the sequence is 104 amino acids long. This will be important later.
- The amino acid sequence is listed using the single letter amino acid code. That is, one letter per amino acid. For example, the amino-terminal amino acid in both cytochrome c’s is glutamic acid, which we would have abbreviated “glu” in Bio 111; here it is “E”. The next amino acid is lysine (“lys” in Bio 111), abbreviated “K”.
- The amino acids are color coded by functional category. For example, aspartic acid (D) and glutamic acid (E) both have (-) charged side chains and are both colored purple.
- The computer program has done its best to match up identical amino acids. Any places where there are differences are shown by white spaces in the purple “Quality” bar under the amino acid sequences. In this case, there are two differences between cytochrome c from cow and donkey:
 - Amino acid #60 in cow cytochrome c is G (glycine); amino acid #60 in donkey cytochrome c is K (lysine).
 - Amino acid #89 in cow cytochrome c is G (glycine); amino acid #89 in donkey cytochrome c is T (threonine).

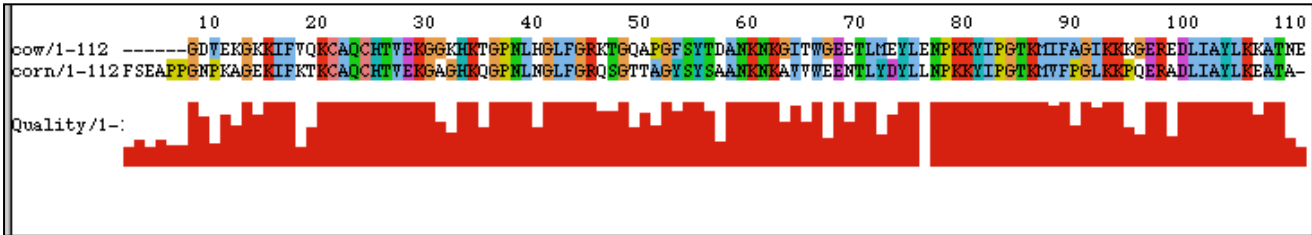
From this, we can conclude that there are two amino acid differences between the cytochrome c’s of cow and donkey. We would then say “cow and donkey differ by 2 substitutions”.

7) Using this technique, find the number of substitutions between your two closely-related organisms. Save this number for later.

8) Choose a third, more distantly-related organism and find the number of substitutions between it and your two original organisms. This will take two separate runs of the program.

I chose corn as my distantly-related organism. Here are the results I got:

• corn vs. cow:

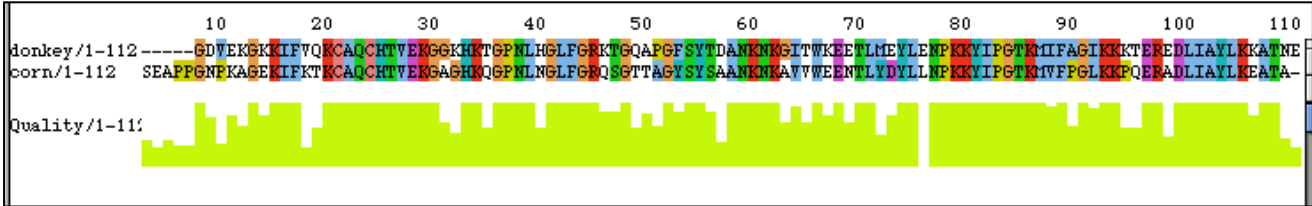


Counting all the places where the sequences don't match:

- Don't forget to count the dashes at the start of the sequence (in this case, there are 5)
- anyplace where the "Quality" bar isn't at its full height (but you can't always see small drops in the "Quality" bar; so you should look at the amino acid sequence carefully)

In this case, there are 44 substitutions out of 112 amino acids.

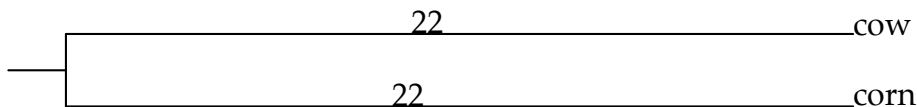
• corn vs. donkey:



Counting all the places where the sequences don't match (see notes above), there are 40 substitutions out of 112 amino acids.

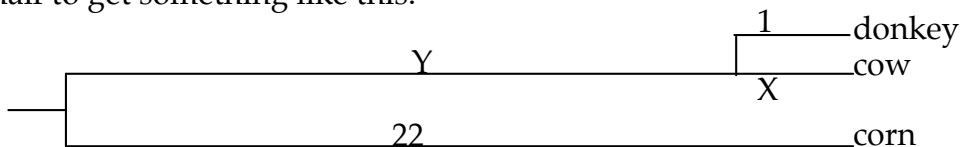
9) Make a phylogenetic tree of your three organisms based on the substitution data. Here is a simple way:

- i) Take the most distantly-related organisms, in this case cow and corn. Make a tree with 2 branches, each 1/2 the number of substitutions long, in this case 44/2 or 22 each.

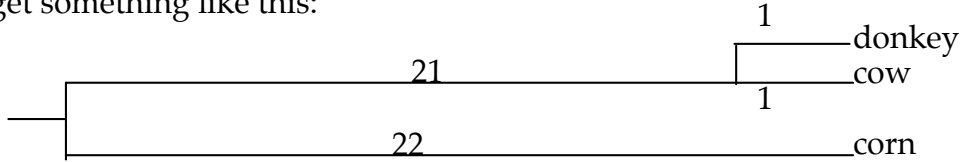


Note that the total distance between cow and corn is $22 + 22 = 44$.

- ii) Now take the more closely-related organism and add it as a branch off of its closely-related partner. In this case, donkey & cow differ by 2. Again, split the difference in half to get something like this:

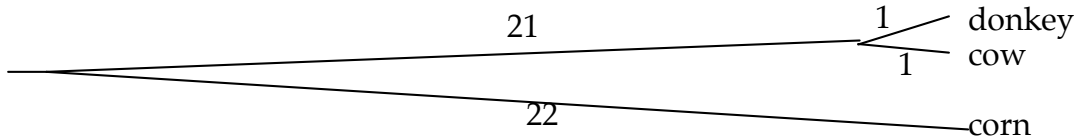


iii) But what about the “X” and “Y”? Since the distance between cow and donkey must be 2, $X + 1$ must = 2. Therefore $X = 1$. Since the total length from the branch at the left to cow must equal 22 and $X = 1$, $Y = 22 - X$ or $22 - 1$, or 21. This gives the final tree: get something like this:



There are a couple of things to notice about this tree:

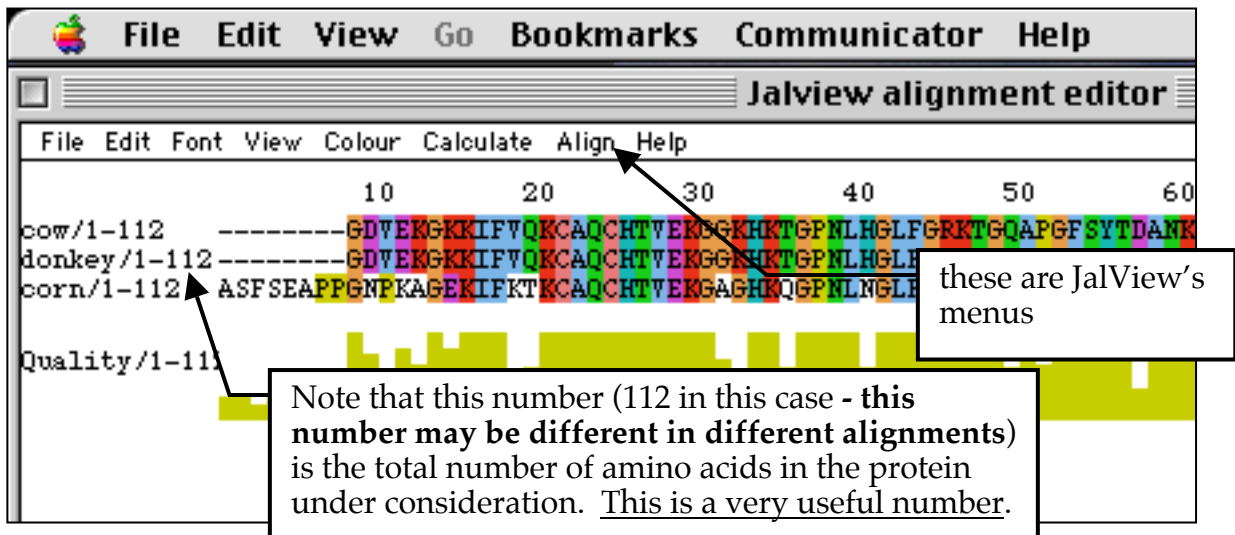
- The lengths of the vertical lines are not counted in the branch lengths. Therefore it is identical to this tree:



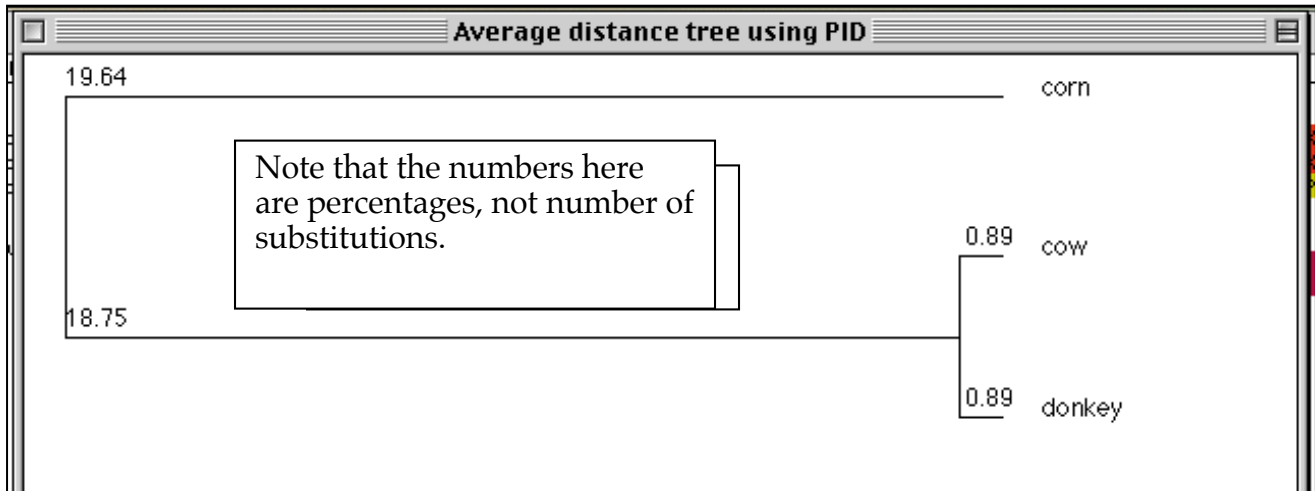
- It is approximate! The distance from donkey to corn should be 41 substitutions (as measured from the sequences) but the tree shows it as 44. Sometimes, it comes out like this and sometimes the numbers add up properly. This is what we call “close enough for government work”.

10) Check the tree you made by having the program calculate it for you.

- Go back to the “Tree constructor” page.
- Select your three creatures and click “Calculate Tree”.
- Click “JalView”.
- When the window appears,



- From JalView’s “Calculate” menu, select “Calculate Average Distance Tree using PID”. Again, be patient. Set the “Font Size” to 12 and check “Show Distances” (these controls are near the bottom of the window) You will get a tree like the one on the next page:



You can roughly check the numbers using the following calculations. The numbers are % difference, so the actual *number* of substitutions = (% difference) x (total number of amino acids).

- the top branch = 19.64% = 0.196. The number of substitutions would be $0.194 \times 112 = 22$ (which is close to the 20.5 in my tree)
- the bottom fork = 0.89% = 0.0089. The number of substitutions would be $0.0089 \times 112 = 1$ (which exactly matches my tree)

* This number may be different in different alignments.

Now that you have seen what the computer does 'behind the scenes', you can leave the hard and boring work to the computer for the rest of the lab.

Part II: Use the Tree Constructor to draw a phylogenetic tree for 5 organisms of your choice and use the rough rule "1 change per 21 million years" to put approximate dates (in Ma) on your tree.

a) Look in the list at the end of this section of the lab manual. Find 5 organisms of your choice. Choose 4 that are from the same kingdom and one from a different kingdom as an "Outgroup organism". Having a distantly-related outgroup organism makes it more likely that the program will give a meaningful tree (the reasons why this is so are beyond the scope of Bio 112).

b) Select the 4 "Main Tree Organisms" as you did previously. Use shift-click to select more than one organism at a time. If you want to select non-adjacent organisms in a list, use apple-click. Once you have made your selections, click the "Calculate Tree" button.

In the example below, I selected:

- Main tree: carp
 chicken
 Chimpanzee
 Cow
 (all of these are vertebrates)
- Outgroup: Corn
 (this is very different from a vetebate!)

c) After a few minutes, you will get a screen like this:

You selected the following creatures:
 Main Tree Organisms: carp, chicken, chimpanzee, cow,
 Outgroup: corn

Tree based on number of substitu

Click on the button below to view the sequences in the JalView multiple alignment editor and to see the tree based on number of substitutions.

Be sure these are the right creatures. If not, scroll down until you see the selection lists and try

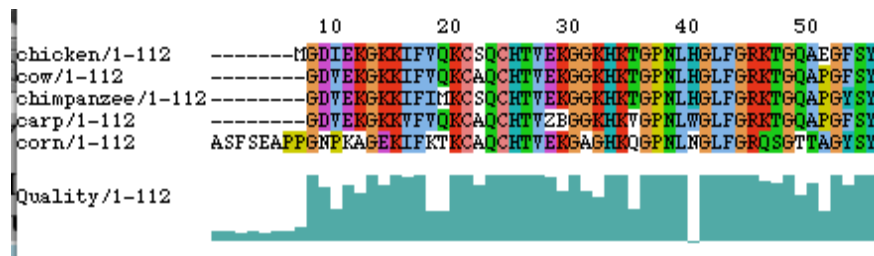
Click here to see the tree calculated based on sequence divergence.

JalView

Sort by group
 Sort by tree order
 Remove redundancy

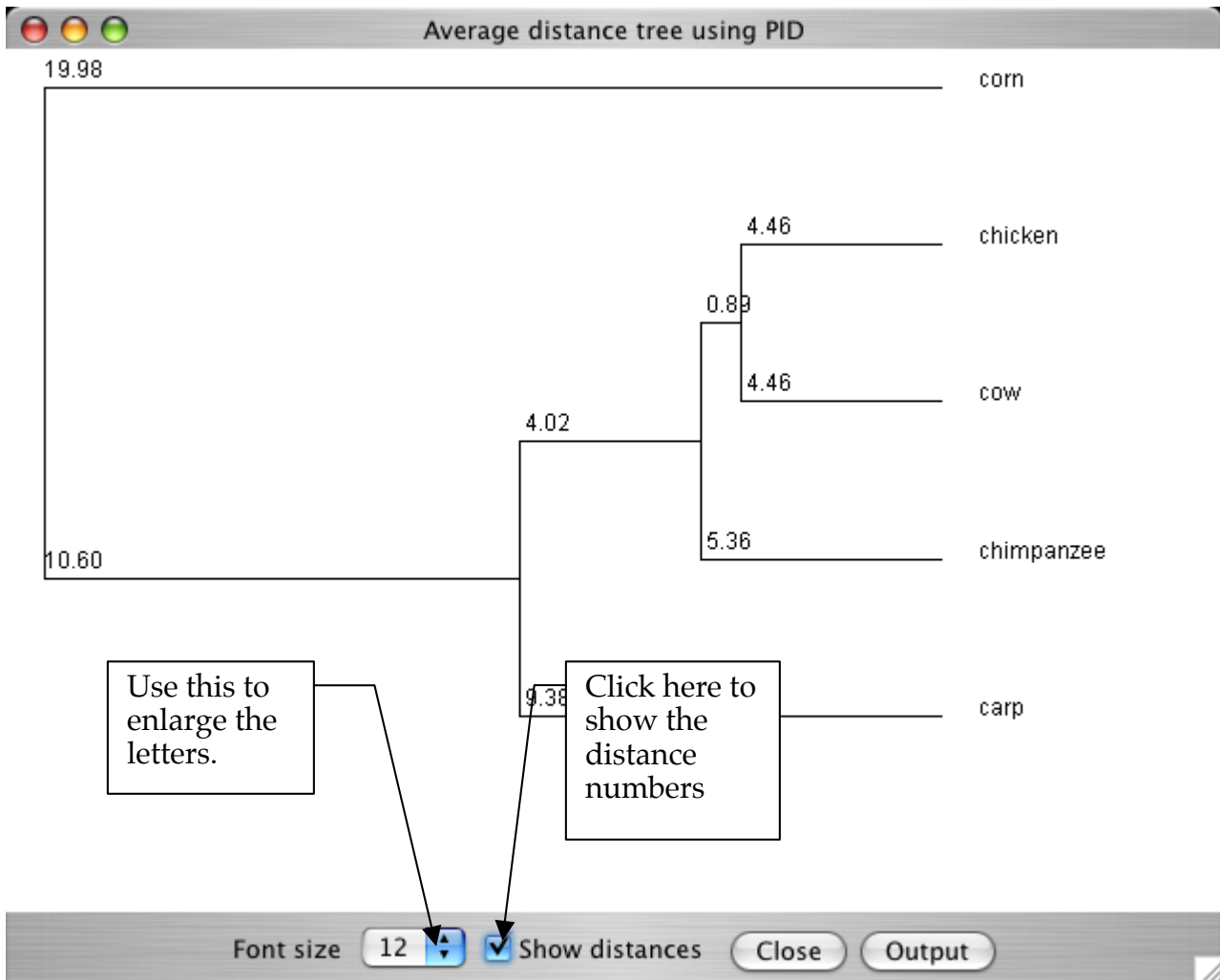
[The only time you should really worry is if you get a message like “server not responding” at this point. In this case, contact Brian White ASAP.]

d) Click the “JalView” button to see the tree calculated based on sequence divergences. (Note that if this is the first time that you have made a tree since Safari was started, it will take a while to load and start the JalView part of the program. You will see messages in the bottom of Safari’s window like “starting Java” and “loading...” please be patient.) You will get a screen like this:



**Note that the dashes “-” above are “gaps” in the alignment - places where a mutation resulted in a *missing* or *added* amino acid. One interpretation of the figure above is that a mutation in an ancestor of corn added several amino acids to the N-terminus of the protein.

e) From JalView’s “Calculate” menu, select “Calculate Average Distance Tree using PID”. Again, be patient. Set the “Font Size” to 12 and check “Show Distances” (these controls are near the bottom of the window). You will get a tree like the one on the next page:



The tree gives the numbers needed to find the approximate date of the last common ancestor of these creatures. You should put approximate dates on your tree using the calculations described on page MolPhyl-16.

f) Unfortunately, you cannot print this out; you will have to copy it down by hand. Do not have the program mail it to you, that feature does not work.

g) Close the JalView windows by clicking the box in the upper left of each JalView window. This should return you to the window shown in step (c).

Part III: Compare trees generated using Sequence Divergence with those generated using Parsimony

a) Now, scroll down to the green area where it says, "Tree Constructed using Parsimony Analysis". It should look something like this:

```
One most parsimonious tree found:

      +-----chimpanzee
    +--3
    ! ! +----cow
    ! +--4
--1  ! +-chicken
    ! +--2
    !   +-carp
    !
+-----corn
```

*** Note that the "!"s don't mean anything - they are just vertical lines. Also, the numbers ("1" through "4") also don't mean anything - they just identify the branch points.

This shows a slightly different result than the one generated using Sequence divergences. Here, the chimp is the most distantly-related vertebrate rather than the carp.

So, which is the "right" tree?

In an ideal world, both methods would give the same answer, more or less. In the real world, things are more complicated. If you were doing this 'for real' (as a scientist trying to draw conclusions about things based solely on this evidence), you would look at more data. Typically, this involves comparing the protein or DNA sequences from many genes in each of the organisms you are interested in. Using only one gene can lead to problems. For example, the cytochrome c sequences of human and chimp are *identical*. Based only on this data, you would conclude that humans and chimps were indistinguishable. This is why we ask that in your lab report, you cite data from more than just this program when drawing conclusions.

In the end, with the limited data and tools available in this lab, the method you choose depends on what you want to know. In general, we would hope that both methods would give similar results. If they don't, then use the following general rule:

- Use Sequence Divergence to find approximately when organisms had a common ancestor.
- Use Parsimony to find out which organisms are more closely-related to each other.

b) To build another tree, click Safari's "back" button twice to return to the "Construct a Tree" screen.

Part IV: Phylogenetic Questions

4) Using these techniques, answer one of the following questions; you should choose the method (Sequence Divergence or Parsimony) that best suits the question you are asking.

- a) Is a Schreibers's long-fingered bat a bird or a mammal?
- b) Is a California grey whale a fish or a mammal?
- c) We will talk about monocots and dicots later in the course; are they distinct groups of plants?
- d) What is a cuckoo-pint? (What kingdom is it in?)
- e) What is a ramtil? (What kingdom is it in?)
- f) What is a love-in-the-mist? (What kingdom is it in?)
- g) A question that you have thought up (it must be approved by your TA in advance).

For example:

1. When was the last common ancestor of X and Y?
2. Which of these organisms are more closely-related?

Note that you will have to compare your results with what is known about these organisms in your lab report (see below), so choose your questions wisely.

Hint: To answer these questions properly, you should use at least two members of each group you are testing and one distantly-related 'outgroup'. The example shown is the correct way to answer the question "is a human a mammal or a plant". The analysis included 6 organisms:

- the organism being examined: human
- two organisms from one test group = 2 mammals = rat & horse
- two organisms from the other test group = 2 plants = corn & cauliflower
- one distantly-related organism: fungus *Aspergillus niger*

Your analyses must use this form, when appropriate. Check with your TA if you have questions.

Part V: Marine Mammals II

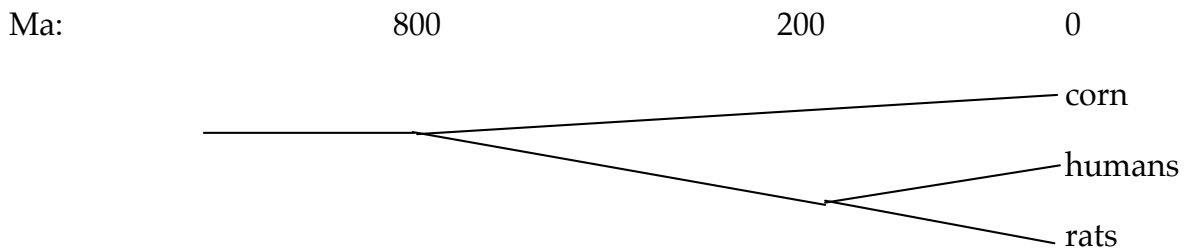
You will answer the two questions from HMNH-8, this time using molecular phylogenetic data. See under "Lab Report" for details. You should do this at home.

Lab report:

- Must be typed; handwritten reports will not be accepted. Hand-drawn and labeled drawings are fine.
- Due at the start of the lab session you are currently in during the week specified on the syllabus. This is a firm deadline.
- Although you will perform these activities as a group, each member of the group must turn in an individual lab report. Each person's report must be in his or her own words as much as possible.
- Your lab report must contain answers to the following 5 questions:

Part II: Draw a phylogenetic tree for 5 organisms of your choice

- 1) The organisms you chose and their kingdom and phylum.
- 2) The tree you constructed using Sequence Divergences with the approximate dates shown. For example, from the data given previously (you tree would have 2 more organisms):



- 3) Compare your tree with the actual values from *Campbell* as best you can. You should compare **both** the actual numbers (which will probably be off at least a little) and the general relationships (which is closer to which). Also compare this tree to the one generated using Parsimony.

Cite your sources. For the above example: "*Campbell* page xxx {you would have to give the actual page #s} puts the plant/ animal split at about 2100Ma - this is not very close to the 800Ma we found. *Campbell*, p. yyy says 'mammal-like synapsids emerged... 200 Ma' We put humans and rats as having a last common ancestor about then (when most major groups of mammals may have diverged), which is probably pretty close." In your lab report, you would expand on this slightly to make a more coherent answer.

Part IV: Phylogenetic Questions

- 4) Answer one of the questions listed. Explain your reasoning and your choice of method (Sequence Divergence or Parsimony). Your answer must include a phylogenetic tree and your conclusions based on that tree. You should also mention if the two methods gave different trees. Your conclusions should hopefully be right but do not have to be right; they must be based on your data, though. You **must also** compare your findings to what is known about the organisms from other sources; you **must also** cite your sources. For example, if you chose question 4b, you should state your results along with something like: "According to *Campbell* (page 725), whales are members of the mammalian order cetartiodactyla and are therefore considered to be mammals. This agrees (or does not agree) with our findings which showed that...."

There is one more question on the next page!!

Part V: Marine Mammals II

5) You answered these two questions in the HMNH lab based on skeletal and morphological data and again in the Skulls lab based on more morphological data. In this part, you will use molecular data to look at the same questions. You should use the link on the On-Line Lab Manual to the “Mammalian Tree Constructor” <http://www.securebio.umb.edu/cgi-bin/COITreeConstructor.pl> This will provide you with a large set of protein sequences from different marine and terrestrial mammals. In this case, the protein is Cytochrome Oxidase II from the mitochondria of the different animals. This protein evolves more rapidly than Cytochrome c, so it is more useful for resolving more recent evolutionary events like the divergence of mammalian groups.

Answer the two questions below using data you gather from the “Mammalian Tree Constructor”. Since the “molecular clock” for cytochrome oxidase II is not well-calibrated, you should use **only parsimony analysis** when answering these questions.

You may need to consult *Campbell* or other sources to determine the mammalian orders to which various animals belong.

WARNING do not try to make a tree with all the organisms. It will take the server forever to compute and it will be so complex that it will be useless for analysis. You should play around to find the most informative small set of mammals for your analysis.

a) How many major different groups of marine mammals are there? A full-credit answer to this question consists of three parts:

- The number of groups of marine mammals that you have determined.
- An explanation of why you chose the groups that you chose. We are not interested in the “right” answer here; just a well-reasoned argument based on your data. Show a parsimony tree that supports your conclusion and explain your reasoning briefly.
- Which of the marine mammals belong to each group? Your answer should include at least two members of each group.

b) Which is the closest living land relative of a seal? Seals evolved from land-dwelling ancestors. Although that ancestor is now extinct, it has modern-day descendants. Based on a phylogenetic tree that you construct, you must decide which order of land mammals this ancestor came from. *Campbell* page 725 has a list of all orders of mammals.

A full-credit answer to this question has two parts:

- The order of land mammals that you think is most closely-related to the land ancestor of seals. Choose from the list below.
- An explanation of why you chose that order. Again, we are not interested in the “right” answer; just an informative tree (one that shows seals’ close relatives and some groups that are not closely-related) and a well-reasoned argument based on your tree.

All of the orders of land mammals can be found in the “Mammalian Tree Constructor” **except:**

- Xenarthra
- Hyracoidea

In each part, we are not interested in the correct answer; we are interested in the *data* you cite and your *argument* based on that data. The more specific about the data you are and the more clear your argument is, the more credit you will get.

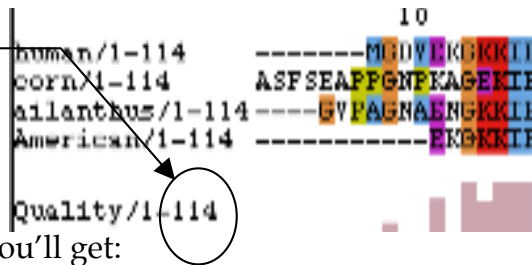
Calculating time since last common ancestor based on JalView's data.

Note that JalView gives the % divergence between two sequences, **not** the number of substitutions. Here is how to use the numbers that JalView puts on a tree to put dates on a phylogeny.

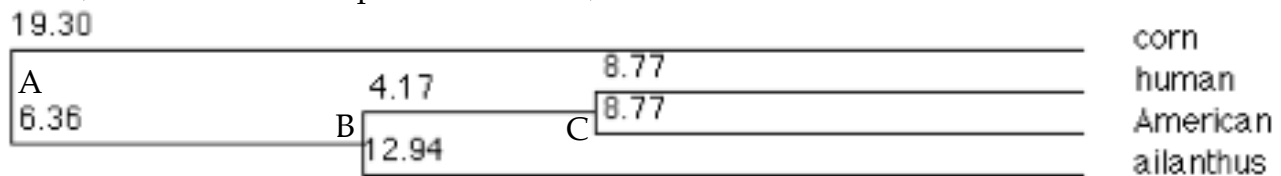
Example: tree of Human, American Alligator, Ailanthus silkmoth, and corn.

1) Construct the tree & click the JalView button. You'll get:

You'll need this number – it's the number of amino acids in the alignment. In this case, 114. **It may be different in different alignments!** Write it down.



2) Have JalView calculate the average distance tree. You'll get:
(I have labeled the splits A, B, and C)



• Find the date of the last common ancestor of corn and the ailanthus silkmoth (split A).

a) The distance from ailanthus & corn is the sum of the lengths of all the branches between them.

$$12.94 + 6.36 + 19.30 = 38.6; \text{ this is the \% divergence.}$$

b) Convert the % divergence to number of substitutions. Multiply % divergence by # of amino acids

$$\text{(from step 2). \# substitutions} = 0.386 \times 114 = 44.0 \text{ substitutions.}$$

c) Correct the number of substitutions by the formula:

$$\text{corrected subs} = -100 \left(\ln \left(1 - \frac{\text{raw substitutions}}{100} \right) \right)$$

$$\text{in this case : corrected subs} = -100 \left(\ln \left(1 - \frac{44}{100} \right) \right) = -100(\ln(0.56))$$

$$= -100(-0.5798) = 57.98 \text{ which rounds to } 58.0$$

d) Convert the corrected divergences to Ma. Use the rule that 1 substitution = 21Ma.

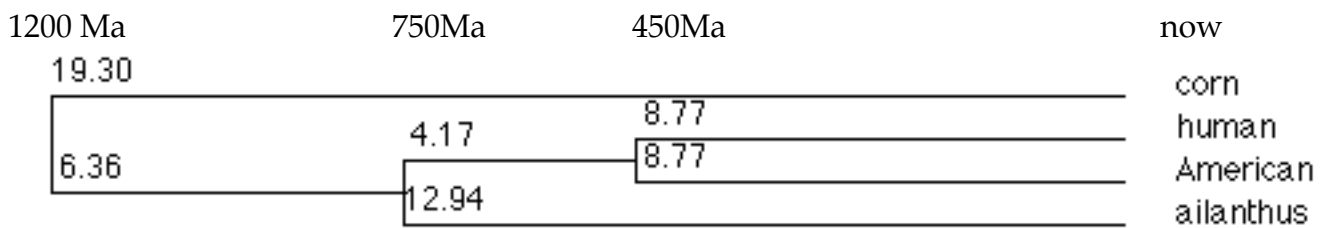
$$\text{In this case: } 58.0 \times 21 = 1218 \text{ Ma ; round this to } 1200\text{Ma.}$$

For example:

- Find the date of the last common ancestor of humans and the ailanthus silkmoth (split B).
 - a) distance = $8.77 + 4.17 + 12.94 = 25.88$;
 - b) # of substitutions = $0.2588 \times 114 = 29.5$
 - c) corrected # of substitutions = 34.95;
 - d) $34.95 \times 21 = 733.95$ or roughly 750 Ma

- Find the date of the last common ancestor of humans and the alligators (split C).
 - a) distance = $8.77 + 8.77 = 17.54$;
 - b) # of substitutions = $0.1754 \times 114 = 20.0$
 - c) corrected # of substitutions = 22.31;
 - d) $22.31 \times 21 = 468.51$ or roughly 450 Ma

⇒ Thus, the correctly-labeled tree would be:



Tables of Organisms:

- Sorted by **kingdom, phylum, etc.**

<u>kingdom</u>	<u>phylum</u>	<u>subgroup</u>	<u>name</u>
animal	annelida		earthworm
animal	chordata	?	California gray whale
animal	chordata	?	Schreibers's long-fingered bat
animal	chordata	mammals	Arabian camel
animal	chordata	mammals	chimpanzee
animal	chordata	mammals	cow
animal	chordata	mammals	dog
animal	chordata	mammals	donkey
animal	chordata	mammals	eastern gray kangaroo
animal	chordata	mammals	guinea pig
animal	chordata	mammals	hippopotamus
animal	chordata	mammals	horse
animal	chordata	mammals	human
animal	chordata	mammals	llama
animal	chordata	mammals	mouse
animal	chordata	mammals	pig
animal	chordata	mammals	rabbit
animal	chordata	mammals	rat
animal	chordata	mammals	rhesus macaque
animal	chordata	mammals	sheep
animal	chordata	mammals	southern elephant seal
animal	chordata	mammals	spider monkey
animal	chordata	mammals	zebra
animal	chordata		American alligator
animal	chordata		bullfrog
animal	chordata		carp
animal	chordata		chicken
animal	chordata		duck
animal	chordata		eastern diamondback rattlesnake
animal	chordata		emu (bird)
animal	chordata		king penguin
animal	chordata		ostrich
animal	chordata		Pacific lamprey
animal	chordata		pigeon
animal	chordata		Puget Sound dogfish
animal	chordata		skipjack tuna
animal	chordata		snapping turtle
animal	chordata		turkey
animal	chordata		western rattlesnake
animal	arthropoda		monsoon river-prawn
animal	echinodermata		starfish
animal	arthropoda		ailanthus silkworm
animal	arthropoda		fruit fly (<i>D. melanogaster</i>)

• Sorted by **kingdom, phylum, etc.** continued

kingdom	phylum	subgroup	name
animal	arthropoda		house fly (<i>Musca domestica</i>)
animal	arthropoda		desert locust
animal	arthropoda		flesh fly
animal	arthropoda		greenbottle fly
animal	arthropoda		honeybee
animal	arthropoda		horn fly
animal	arthropoda		Mediterranean fruit fly
animal	arthropoda		tobacco hornworm
animal	mollusca		brown garden snail
animal	nematoda		Nematode
bacteria	proteobacteria		Bacterium: <i>Desulfovibrio vulgaris</i>
fungi	ascomycota		<i>Aspergillus nidulans</i>
fungi	ascomycota		<i>Aspergillus niger</i>
fungi	ascomycota		<i>Neurospora crassa</i>
fungi	basidiomycota		smut fungus
plantae	angiospermae	dicot	castor bean
plantae	angiospermae	dicot	cauliflower
plantae	angiospermae	dicot	China jute
plantae	angiospermae	dicot	hemp
plantae	angiospermae	dicot	mung bean
plantae	angiospermae	dicot	nasturtium
plantae	angiospermae	dicot	oriental sesame
plantae	angiospermae	dicot	parsnip
plantae	angiospermae	dicot	potato
plantae	angiospermae	dicot	pumpkin
plantae	angiospermae	dicot	rape
plantae	angiospermae	dicot	sea-island cotton
plantae	angiospermae	dicot	spinach
plantae	angiospermae	dicot	sunflower
plantae	angiospermae	dicot	tomato
plantae	angiospermae	monocot	buckwheat
plantae	angiospermae	monocot	corn
plantae	angiospermae	monocot	leek
plantae	angiospermae	monocot	rice
plantae	angiospermae	monocot	wheat
plantae	Ginkgophyta		ginkgo
protocista	chlorophytes		green alga
protocista	ciliates		<i>Tetrahymena pyriformis</i>
protocista	euglenids		<i>Crithidia fasciculata</i>
protocista	euglenids		<i>Crithidia oncopelti</i>
protocista	euglenids		<i>Euglena gracilis</i>
protocista	euglenids		<i>Euglena viridis</i>
?	?	?	love-in-a-mist
?	?	?	ramtil
?	?		cuckoopint

- Sorted by **name**:

kingdom	phylum	subgroup	name
animal	arthropoda		ailanthus silkmoth
animal	chordata		American alligator
animal	chordata	mammals	Arabian camel
fungi	ascomycota		Aspergillus nidulans
fungi	ascomycota		Aspergillus niger
bacteria	proteobacteria		Desulfovibrio vulgaris
animal	mollusca		brown garden snail
plantae	angiospermae	monocot	buckwheat
animal	chordata		bullfrog
animal	chordata	?	California gray whale
animal	chordata		carp
plantae	angiospermae	dicot	castor bean
plantae	angiospermae	dicot	cauliflower
animal	chordata		chicken
animal	chordata	mammals	chimpanzee
plantae	angiospermae	dicot	China jute
plantae	angiospermae	monocot	corn
animal	chordata	mammals	cow
protocista	euglenids		Crithidia fasciculata
protocista	euglenids		Crithidia oncopelti
?	?		cuckoopint
animal	arthropoda		desert locust
animal	chordata	mammals	dog
animal	chordata	mammals	donkey
animal	chordata		duck
animal	annelida		earthworm
animal	chordata		eastern diamondback rattlesnake
animal	chordata	mammals	eastern gray kangaroo
animal	chordata		emu (bird)
protocista	euglenids		Euglena gracilis
protocista	euglenids		Euglena viridis
animal	arthropoda		flesh fly
animal	arthropoda		fruit fly
plantae	Ginkgophyta		ginkgo
protocista	chlorophyta		green alga
animal	arthropoda		greenbottle fly
animal	chordata	mammals	guinea pig
plantae	angiospermae	dicot	hemp
animal	chordata	mammals	hippopotamus
animal	arthropoda		honeybee
animal	arthropoda		horn fly
animal	chordata	mammals	horse
animal	arthropoda		housefly
animal	chordata	mammals	human
animal	chordata		king penguin

- Sorted by **name** continued:

kingdom	phylum	subgroup	name
plantae	angiospermae	monocot	leek
animal	chordata	mammals	llama
?	?	?	love-in-a-mist
animal	arthropoda		Mediterranean fruit fly
animal	crustacea		monsoon river-prawn
animal	chordata	mammals	mouse
plantae	angiospermae	dicot	mung bean
plantae	angiospermae	dicot	nasturtium
animal	nematoda		Nematode
fungi	ascomycota		Neurospora crassa
plantae	angiospermae	dicot	oriental sesame
animal	chordata		ostrich
animal	chordata		Pacific lamprey
plantae	angiospermae	dicot	parsnip
animal	chordata	mammals	pig
animal	chordata		pigeon
plantae	angiospermae	dicot	potato
animal	chordata		Puget Sound dogfish
plantae	angiospermae	dicot	pumpkin
animal	chordata	mammals	rabbit
?	?	?	ramtil
plantae	angiospermae	dicot	rape
animal	chordata	mammals	rat
animal	chordata	mammals	rhesus macaque
plantae	angiospermae	monocot	rice
animal	chordata	?	Schreibers's long-fingered bat
plantae	angiospermae	dicot	sea-island cotton
animal	chordata	mammals	sheep
animal	chordata		skipjack tuna
fungi	basidiomycota		smut fungus
animal	chordata		snapping turtle
animal	chordata	mammals	southern elephant seal
animal	chordata	mammals	spider monkey
plantae	angiospermae	dicot	spinach
animal	echinodermata		starfish
plantae	angiospermae	dicot	sunflower
protocista	ciliates		Tetrahymena pyriformis
animal	arthropoda		tobacco hornworm
plantae	angiospermae	dicot	tomato
animal	chordata		turkey
animal	chordata		western rattlesnake
plantae	angiospermae	monocot	wheat
animal	chordata	mammals	zebra

Planting c-fern Spores

Purpose:

- to set up for the plant labs later in the semester

Introduction

Later in the semester, we will talk extensively about plant life cycles. These are surprisingly strange, involving spores, haploids, and gametes produced by mitosis (!). One way to make this challenging material clear is to follow the life cycle of a simple plant completely. Don't worry about the terminology for now. The lab is intended to plant (pun intended) some of these ideas and terms in your mind for later reference.

Ferns like the c-ferns you will be working with are dispersed by spores – haploid cells produced by meiosis – instead of seeds. In fact, ferns do not have seeds at all. Today, you will observe where the spores in this fern are produced (called a *sporophyte*) and plant some of them on nutrient medium. You will then observe them in two or three weeks when they have grown into *gametophytes*.

Procedure

1. Be sure to look at the sporophyte samples to see where the spores are made. The sporophyte looks like a fern; the spores are tiny black dots.
2. Put on gloves.
3. Label your plate of c-fern media on the bottom using tape and marker; put your name, TA & section.
4. Wipe down your work area with ethanol and a paper towel.
5. The TA will tap the tube of c-fern spores on the table to make sure they are in the bottom of the tube. He/she will add 1.25 ml sterile water to the tube and invert the tube 2-3 times to make sure the spores are wetted and suspended.
6. Each group will take 3 drops of suspension from this tube and sow their c-fern spores onto their plates of media.
7. When your group is ready to plant its spores, rinse the blue plastic spore spreader in the jar of alcohol and shake off any excess (not in the direction of any people). Stand it, spreader side up, in the beaker to dry. Make sure the spreader is dry before you use it.
8. Open the tube of spores and with your sterile pipet bubble some air into the tube to resuspend the spores. Take up some of the suspension in your pipet and drop 3 drops of suspension onto your media. Lift the lid up only as high as you need to to put the drops in. Holding the lid over the plate with one hand (or another student can hold the lid over the plate) spread the drops of suspension over the surface of the media using your blue spreader, turning the plate as you spread. Continue this turning of the plate and spreading motion until all of the liquid has been absorbed into the media.
9. Place your covered, labeled plate into the black flat on the counter. Do not tape the cover to the plate. C-fern spores need a temperature of ~28° C to germinate and grow. In 2 weeks, these c-ferns will be ready for fertilization.

