



5. Remember that the general pathway for sequencing SSU rDNA data and then inferring phylogenetic relationships goes along these lines:

DNA extraction → PCR → Sequencing → Alignment → Phylogenetic analysis

One issue that researchers confront is ... what method should be used for inferring phylogenies? Different methods have distinct strengths and weaknesses. The most widely used algorithms (inference methods) include:

- (1) Distance methods: in this case, a parameter called evolutionary distance is calculated. Evolutionary distance represents the percentage of nonidentical sequences between two organisms. These distances are put into a matrix, are corrected for the possibility of multiple changes at any given nucleotide, and then are used to infer a phylogeny in which the branch lengths are proportional to the evolutionary distance between sequences. A brief way to say this: a phylogenetic tree based on distance reflects the percent similarity of sequences.
- (2) Parsimony methods: here, the goal is to arrange organisms on a tree in such a way as to minimize the number of evolutionary changes on the tree.
- (3) (not mentioned in the text) Maximum likelihood, and
- (4) (not mentioned in the text) Bayesian methods.

Students interested in the latter two approaches should feel free to discuss them with me.

**Section 11.6** should resonate with your reading from Ch. 18, and we won't go into further detail here. Make sure, though, that you feel very comfortable with FISH (our lecture notes and the text from Ch. 18 will be sufficient for the exam; the material presented here provides extra detail).

**Section 11.7** also should contain material that is largely review, including the structure of the three-domain system, the position of the domains of cellular life on the tree, etc. We will not be addressing these topics in detail again, as we have covered them fairly extensively. Please look over this section and note the connections with your lecture notes, with additional focus on two points:

6. What organisms that are currently living might be described as primitive? Why or why not?
  
7. Please look at the subsection entitled Eukarya.
  - a. Give an example of a eukaryotic organism.
  - b. What is the 16S gene called in the context of Eukarya?
  - c. The earliest eukaryotes are thought to resemble modern microsporidia and diplomonads (we will meet these in class; one example is the pathogen *Giardia*, which causes gastrointestinal infections in mammals). What do characteristics do these organisms share?

- d. What is distinctive about the cellular structure of microsporidia and diplomonads in the context of endosymbiosis?
  
- e. What major ecological change – at a global scale -- is thought to have triggered the evolutionary radiation of the Eukarya?

**Section 11.8:** again, this is a review – here, the focus is on the major characteristics of the domains. This should resonate with your lecture notes (from Exam 2), and will be revisited in class when we introduce the Eukarya.

**Part III, Section 11.9:** this section will focus on taxonomy – that is, the classification of organisms (in our case, microbes).

- 8. What is taxonomy, and what two major subdisciplines does it incorporate?
  
- 9. Distinguish between taxonomy and phylogeny.

There are two major types of taxonomy: classical taxonomy, and molecular taxonomy.

- 10. **Classical taxonomy** typically relies on phenotypic characteristics regarding morphology, nutrition, physiology, and habitat. More recently, some taxonomists have added GC ratios as a character. How are GC ratios calculated?

Beware! Remember that GC ratios are of limited use, because very different organisms can have the same GC content in their genomes. Alternatively, close relatives may have very different GC content!

- 11. Review table 11.4 and carefully read Figure 11.17 to get a vision of classical taxonomic methods.
  
- 12. **Molecular taxonomy** is also called chemotaxonomy. This approach employs molecular analyses to identify and classify organisms. The three main methods are DNA:DNA hybridization, ribotyping, and lipid analyses.

13. DNA:DNA hybridization: the rationale for this method is that two DNAs are expected to hybridize to one another in proportion to the similarity of their gene sequences. I expect you to understand this rationale based on the reading, and I will cover the 'how it works' portion in lecture. Please do address these two questions:

- a. Is DNA:DNA hybridization most useful for differentiating between closely related, or distantly related, organisms?
- b. Do DNA:DNA hybridization results always agree with results from phenotype screening or SSU rDNA sequencing?

14. Ribotyping: this is a method that goes by the popular name of 'molecular fingerprinting'. In this method, sequences are not generated; instead, DNA is digested using restriction enzymes, and the resulting fragments are separated and probed with an RNA probe. The result is a series of bands on a gel that is unique to a species or stain. The rationale here is that restriction enzymes work at particular sites in a gene; if those sites are missing, the resulting fragments of the gene will be of different lengths and will differ in number – yielding highly distinctive band patterns on a gel following electrophoresis.

- a. What are two benefits of ribotyping, and how are these aspects beneficial?

15. Lipid analyses: the best-known methods here focus on fatty acids (types, and proportional representation of those types) in lipids that comprise the membranes of cells (and outer membranes of Gram-negative bacteria). The idea here is to separate fatty acids into their component methyl esters, and then to examine these using gas chromatography. The output will show the types and amounts of particular fatty acids; this profile can be compared among bacteria to provide an identification tool. See Fig. 11.21 for a nice overview of the method.

- a. What is the nickname of this method, and what does it stand for?
- b. This method is often used in clinical, public health, and food/water inspection applications. What factors should be controlled in order to generate the most reliable results?

16. **Section 11.11.** How are species typically (traditionally) defined for macroscopic organisms?

- a. Why does this species concept not apply particularly well to microbes?
- b. True or false: there's no such thing as a 'species' in bacteria.

17. What term is used to refer to the synthesis of many methods to classify microbes?
18. What level of SSU rDNA similarity is typically used to define species of prokaryotes?
  - a. What is the rationale for using this level of similarity to distinguish between species of microbes?
19. What are the taxonomic divisions of organisms, starting with their domain – and going all the way down to species? (David Poured Chocolate Over Five Green Snakes...)
20. We will talk about bacterial speciation in class, focusing on ecotype selection (see Fig. 11.23 for a good illustration of this principle) and lateral/horizontal gene transfer.
21. **Section 11.12** focuses on nomenclature. What happens when one discovers an apparently new species? A detailed description of the species must be published, along with the proposed name. A viable culture of the organism is deposited in at least two culture collections (e.g., American Type Culture Collection, in Virginia, or the DSMZ, in Germany) where the strain will be frozen, freeze-dried, or otherwise preserved in a living but metabolically suppressed state. If the new species and name are accepted by the community, and if the species is a prokaryote, then it will be listed in *Bergey's Manual* – a major resource for bacteriologists. At the moment, there is no such centralized volume for the species of microscopic eukaryotes.