

Reconstrução de Caráter Ancestral no **Mesquite** (parsimony/ maximum likelihood)

Para iniciar você vai precisar de uma árvore (ex. Galapagos_Finch_Tree) no formato .nex e uma planilha (ex. Galapagos_Finch_Data.txt) no formato .txt com os caracteres/ estados de caráter.

Ancestral reconstruction is often the starting point for comparative analysis. In this exercise, we'll get acquainted with the two main types of methods used for ancestral reconstruction: parsimony and maximum likelihood. To reconstruct ancestral character states using these methods, we'll need to get acquainted with the program **MESQUITE** (<http://mesquiteproject.org/>)



Figure 1 - The beaks of Darwin's finches.

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What did the ancestral Darwin's Finch eat?

We're going to use Mesquite to reconstruct the diet of Galapagos finches. This example was at the center of Schluter et al.'s (1997) pioneering paper titled "Likelihood of Ancestor States in Adaptive Radiation". Darwin's finches are categorized as one of three dietary specialists: granivore, insectivore, or folivore. We would like to know which of these diets characterized the ancestral Darwin's finch. In addition to learning the basics of ancestral reconstruction with Mesquite, we will also explore one important cause for conflict between parsimony and likelihood reconstructions. In particular, we will consider the impact of branch lengths on maximum likelihood reconstructions. Anyone interested in doing ancestral reconstruction should be aware of the serious limitations of this approach, which may lead to dramatically incorrect evolutionary inference; in addition to Schluter et al. (1997), Cunningham (1999) and Losos (1999) are required reading for anyone considering a project involving ancestral reconstruction. As a result of these and other criticisms of ancestral reconstruction, most comparative biologists now favor methods that integrate over all possible reconstructions over those that rely on point reconstructions.

Part One: Generate a Mesquite Project

1. Open Mesquite by double-clicking on the Mesquite icon.
2. Go to the "File" tab and click on "Open".
3. Navigate to the file titled "Galapagos_Finch_Data.txt". This is simply a tab-delimited file generated in Microsoft Excel that contains two columns, one with the names of the finch species and another with a code for their diet (0=granivore, 1=insectivore, 3=folivore). Because this is not a NEXUS file, a pop-up window should appear telling you that you need

to choose an interpreter. Because we're dealing with data that is categorically coded, we're going to select "Simple (categorical data)" and click "OK". When prompted to save this file, save it as "Finch_Diet.nex".

4. At this point, there should be two Mesquite windows open, one called "Mesquite Projects and Files" that lists current projects and associated files and another titled "Character Matrix" that lists the taxa and character states for the finch data.

5. Now that our data is there, we want to get our tree into Mesquite. To do this, go to the "File" tab, click on "Include File..." and open the file called "Galapagos_Finch_Tree" (this is a NEXUS file containing a single tree). Although you won't immediately see a tree after opening this file, you should see a new line called "Tree block: Galapagos Finch Phylogeny" in the list of files in the "Projects and Files" window.

6. To see the tree, you need to go to the "Taxa&Trees" tab and select "New Tree Window" (NOTE: The set of tabs you see on the top of your screen will depend on whether the "Mesquite Projects and Files" or the "Character Matrix" window is active. To see the "Taxa&Trees" tab, the "Character Matrix" window must be active. To make a window active just click on it.). You should be prompted to select the "Source of trees (Tree window)", at which point you should click on "Stored Trees" and click "OK". You should now see a new window that displays the finch tree.

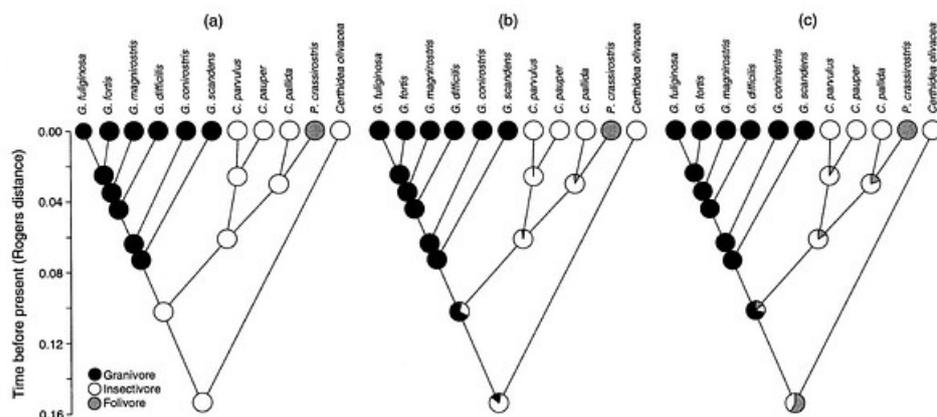


FIG. 1. A phylogeny of 11 of the Galápagos finches based on allozymes (Yang and Patton 1981). The tree is a UPGMA diagram calculated from Rogers's distances. Statistical support for the three groups is fairly strong (ground finches *Geospiza*, tree finches *Camarhynchus-Platyspiza*, and the warbler finch *Certhidea*), but other aspects of tree topology are weakly supported (Stern and Grant 1996). (a) Ancestor states estimated using the method of maximum parsimony (Maddison and Maddison 1992). (b) Maximum-likelihood ancestor states assuming that all transition rates q are equal; $\hat{q} = 3.6$. Area of pies indicate relative support for different ancestor states. (c) Maximum-likelihood ancestor states when transition rates are free to vary.

Figure 1 - From Schluter et al. 1997

Part Two: Reconstruct Ancestral States With Parsimony and Likelihood

7. To reconstruct ancestral states, go to the "Analysis" tab associated with the tree window. From this tab, select "Trace Character History". Select "Stored Characters" and click "OK", then select "Parsimony Ancestral States" and click "OK". Your branches in the tree window should now appear as shaded bars that correspond with the three character states. Also note that the number of steps required for this character's evolution is noted in the "Trace Character" palette (you should observe 2 steps). (If you want to change the colors associated with each character, just double click on the colored rectangles in the "Trace Character" palette).

8. To see this in a slightly different way (which will be useful in making comparisons to the likelihood reconstructions we're about to generate), go to the "Drawing" tab and select "Tree Form" and select "Balls&Sticks".

9. Now, we're ready to do **ancestral reconstruction using maximum likelihood** and to explore the differences between parsimony and likelihood methods. First, go back to step 6 and open a new tree window. Now reconstruct ancestral states on this tree by repeating step 7, but select "Likelihood Ancestral States" instead of "Parsimony Ancestral States". When prompted, select "Current Probability Model". You should see a tree with a pie diagram at each node. The shaded portions of each diagram correspond with the relative likelihood of each character state. You should notice immediately that rather than being unambiguously reconstructed as an insectivore (state 1), ML suggests uncertainty in the character state of the ancestral finch. This is one of the chief benefits of ML - it provides some indication of our confidence in each possible ancestral state. Notice also that the most likely ancestor in this analysis differs from that recovered by parsimony: with ML a granivore ancestor appears more likely than the insectivore ancestor recovered by MP.

10. We now want to use Mesquite to **explore why parsimony and likelihood disagree**. To do this, we'll need to examine branch lengths (recall that likelihood uses branch length data, whereas parsimony does not). To see branch lengths in Mesquite, go to the "Drawing" tab and select "Branches Proportional to Lengths" (NOTE: the branch lengths in this exercise are fabricated and are for illustrative purposes only). Notice that the ancestor of the granivores (state 0) is separated from the ancestor of the clade containing pauper and pallida by a relatively short branch. Is the relatively low probability of a change along this branch responsible for the fact that likelihood prefers a granivore ancestor for the entire radiation?

11. To test question raised above, we can manipulate the length of branches with the "Stretch branch" tool located on the Mesquite toolbar (it's the one with a ruler next to arrows pointing up and down [or you can find it by moving your mouse over the tools and waiting for description to pop-up]). After selecting this tool, click on the ancestor of the granivores and drag this node up (to do this in Mesquite you will actually be dragging more to the left than up). Although the tree is no longer ultrametric, you should be able to see that an insectivore ancestor becomes increasingly more likely as this branch gets longer.

Exemplo retirado de:

http://bodegaphylo.wikispot.org/Basic_ancestral_reconstruction_with_parsimony_%26_maximum_likelihood_in_Mesquite

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