## Expected mutation counts from four-fold degenerate sites.

The third nucleotide in codon acc is four-fold degenerate since aca, acc, acg, and act all encode threonine.

We count the number of *mutations* on branches of the tree, not the final alignment: in tree at right there are three  $c\rightarrow g$  mutations even though four sequences have the mutation.

## Actual counts of mutations at each site.

In tree at right, there is one count of the indicated c→g mutation, which induces an alanine to glycine mutation. The fact that the actual counts are less than the expected counts suggests this mutation is deleterious.



