## Estimate mutation rates at 4-fold degenerate sites.

The third nucleotide in codon acc is 4-fold degenerate since aca, acc, acg, and act all encode threonine.

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

## Determine actual counts of mutations at each site.

In the tree at right, there is one count of the indicated  $c \rightarrow g$  mutation, which induces an alanine to glycine mutation .



