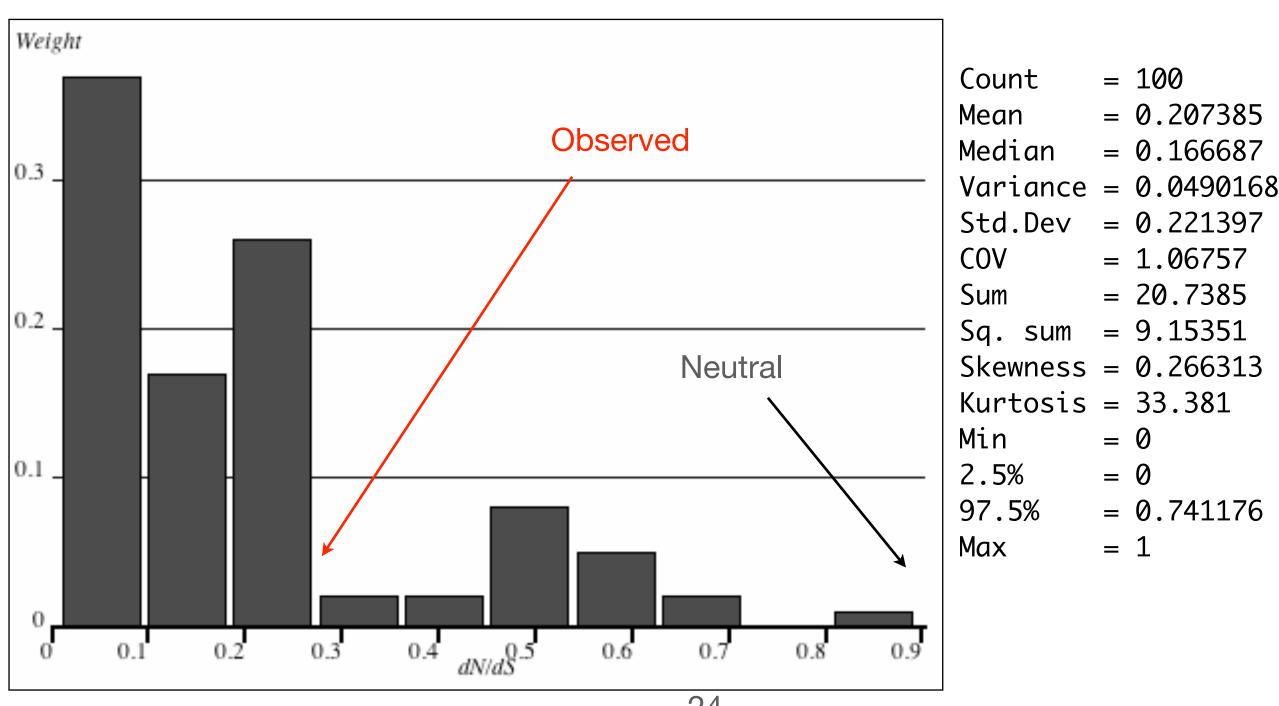
NG86 example

- How reliable is the inference based on only 6 codons?
- Obtain sampling variance via bootstrap (or by limiting approximations)
- In this case, dN/dS is significantly less than 1.0 (p ~ 0.01)





NG86 limitations: multiple substitutions

- How many synonymous and how many non-synonymous substitutions does it take to replace CCA with CAG?
- Assume the shortest path (minimum of 2 substitutions)

```
• CCA (Proline) \rightarrow CAA (Histidine) \rightarrow CAG (Glutamine)
```

- CCA (Proline) \rightarrow CCG (Proline) \rightarrow CAG (Glutamine)
- Average over the two possible paths: 0.5 synonymous and 1.5 nonsynonymous substitutions.
- Intuitively, paths should not be equiprobable, e.g., because it should be more expensive to route evolution through (presumably) suboptimal intermediate amino-acids.