Lecture 17

- Detecting sequence conservation with PhyloHMMs
- PhastCons

PhyloHMMs

- Yang 1995; Felsenstein & Churchill 1996

- Siepel A. *et al.* (2005): Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res.* 15:1034-50
  - basis of PhastCons conservation scores (UCSC genome browser)

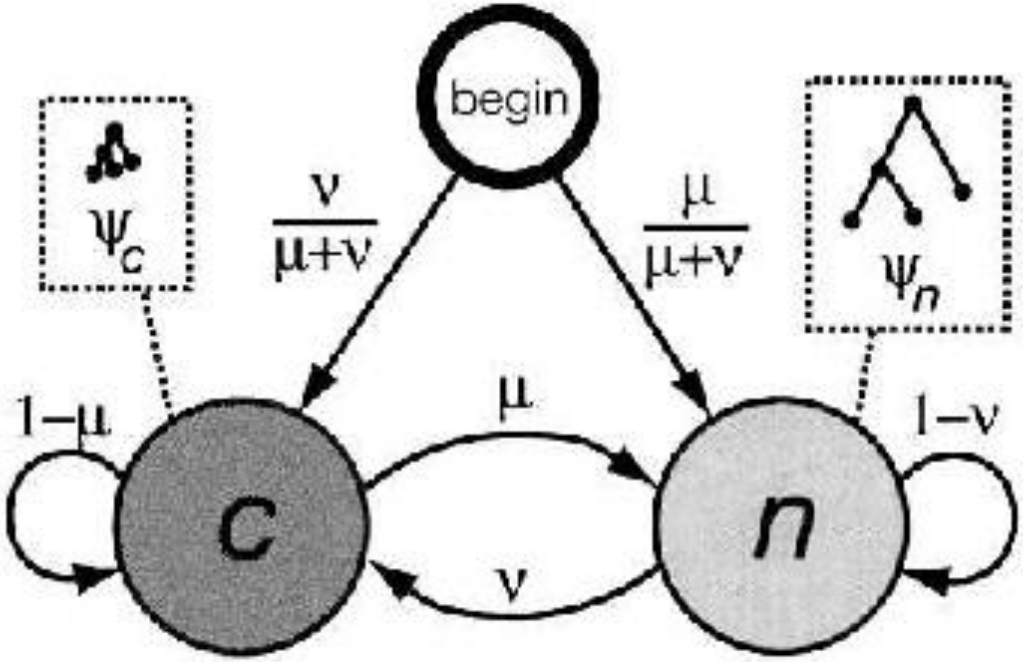
- Goal: starting from multiple genome sequence alignment, identify
  - conserved regions (regions under purifying selection),

  against background of
  - neutrally evolving regions

PhastCons PhyloHMM

- model:
  - 2-state HMM
    - c: conserved state
    - n: neutral (or nonconserved) state
  - emitted symbols are *alignment columns*
  - emission probabilities based on *phylogenetic tree* relating sequences
  - gaps in alignment treated as *missing data*

PhastCons PhyloHMM



$\mu = a_{cn}$

$\nu = a_{nc}$

$$\mathbf{x} = \begin{matrix} \text{TCGCGACATATACGA}\ldots \\ \text{TTGGGGCATGTGGGT}\ldots \\ \text{AGCAGACGTCCGCAA}\ldots \end{matrix}$$

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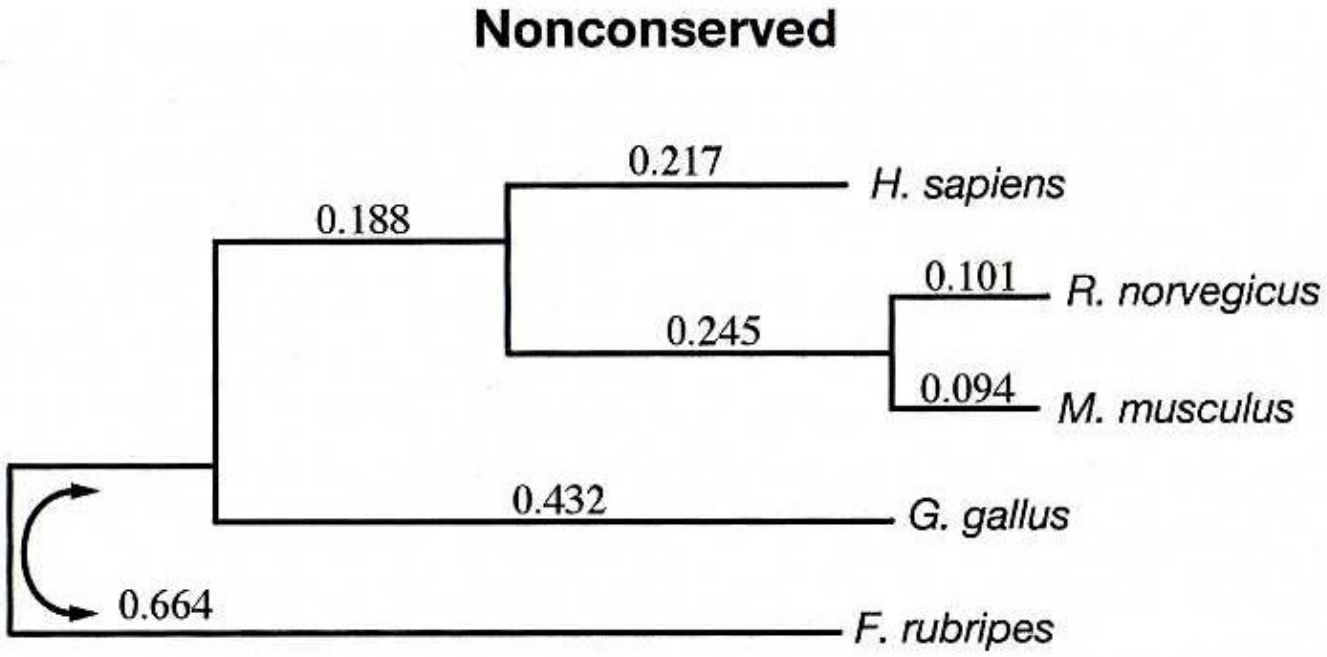
Nonconserved
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H. sapiens
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R. norvegicus
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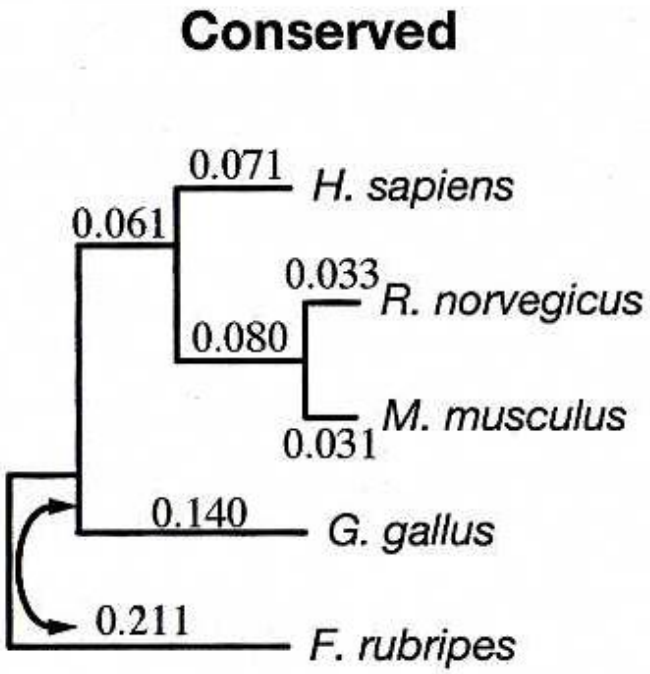
0.094
M. musculus
0.432
G. gallus
0.664
F. rubripes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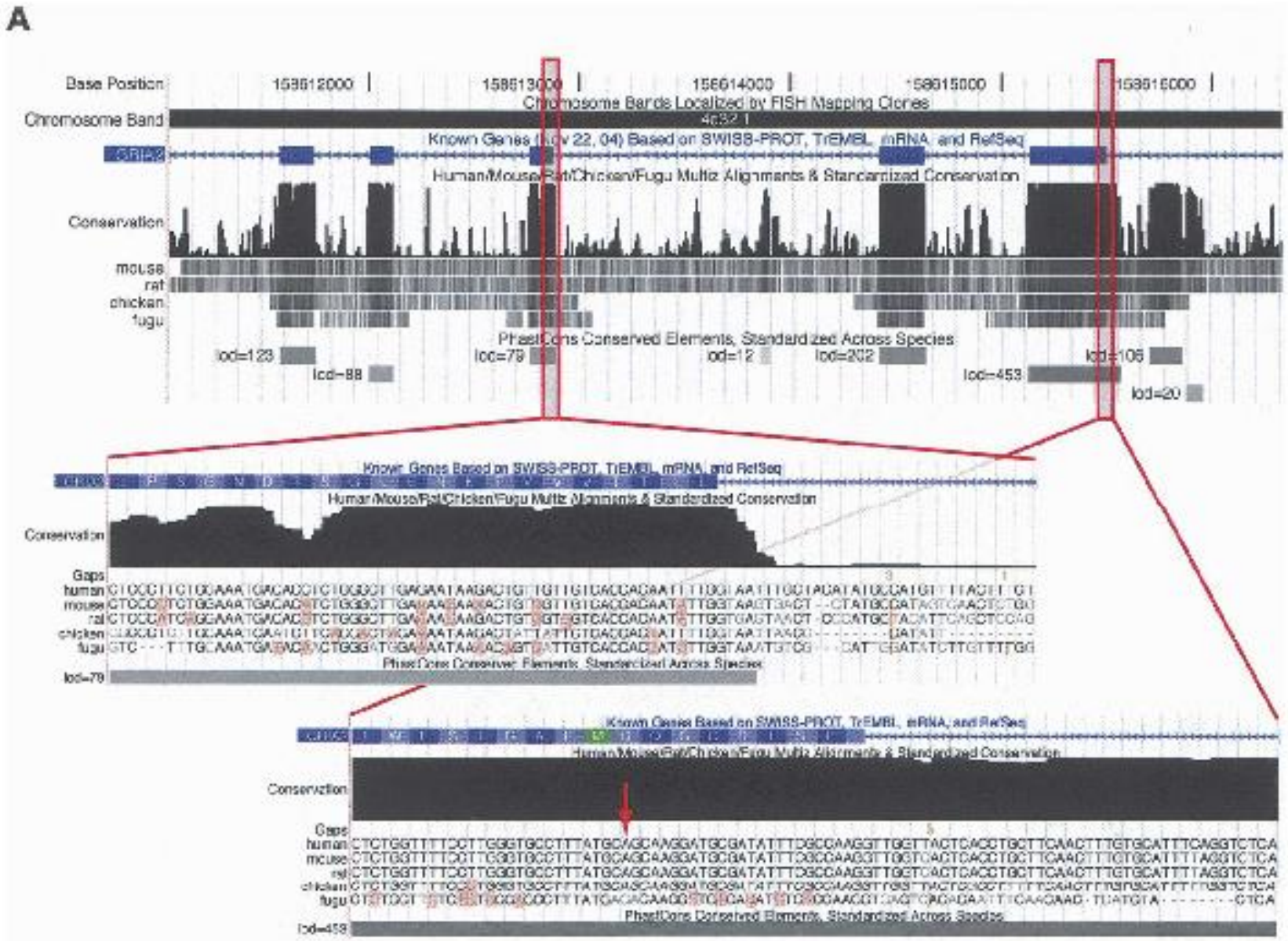
Siepel *et al* evolutionary model

- single, reversible, infinitesimal mutation process across tree
- branches differ only in their lengths
- selection strength same across tree and sites

- branch lengths:
  - Expected # substitutions/site over corresponding evolutionary time period
  - for neutral state, should reflect underlying mutation rate
  - for conserved state: mutation rate $\times$ scaling factor ρ
    - ρ = frac of mutations that escape purifying selection
    - $\rho \approx .33$ (for vertebrates)



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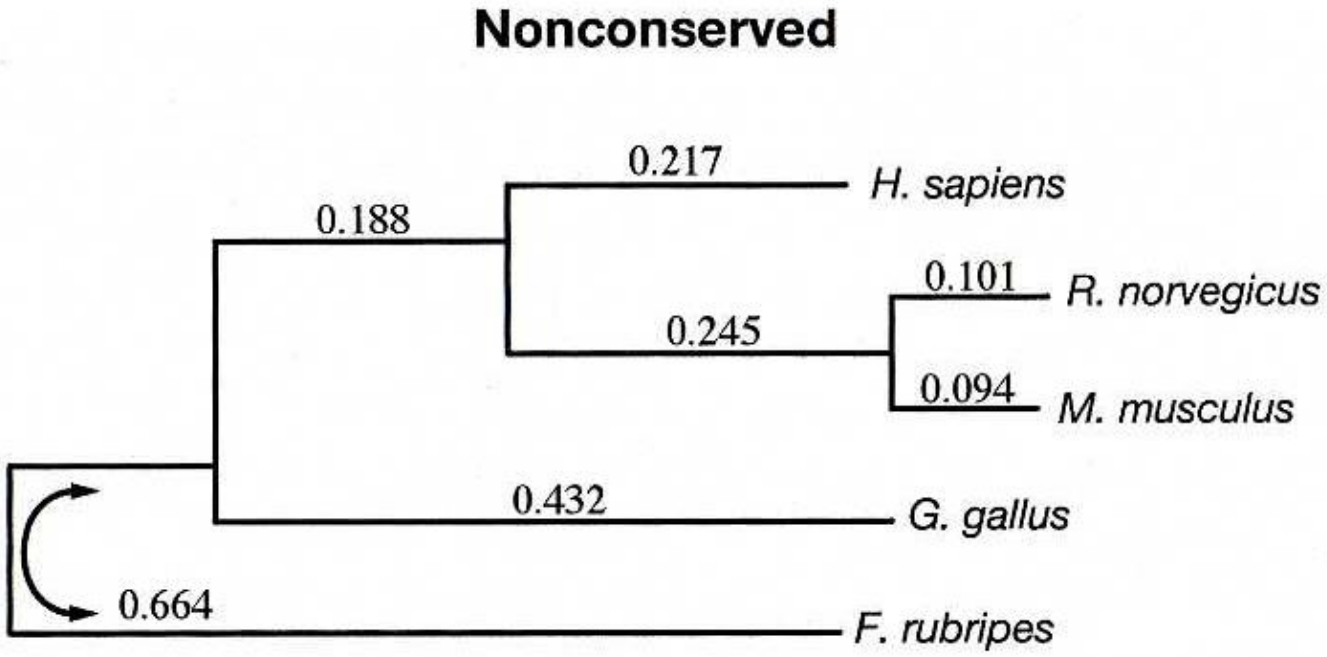
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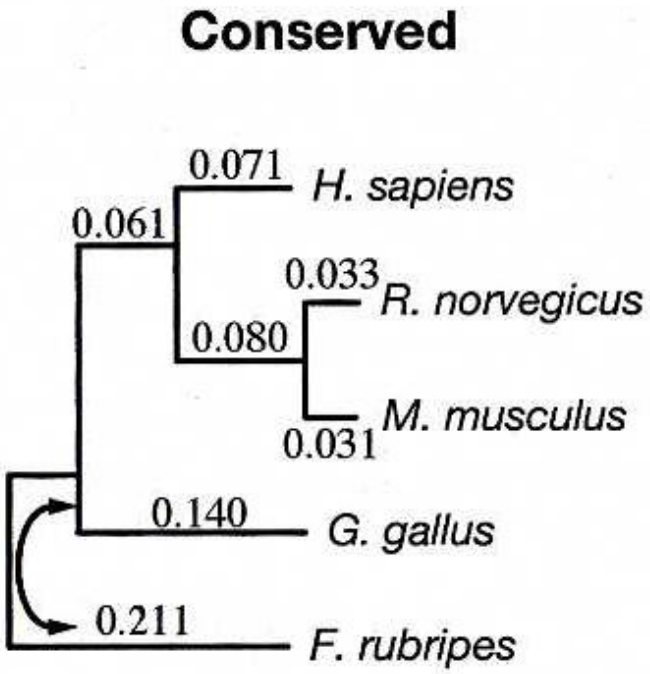
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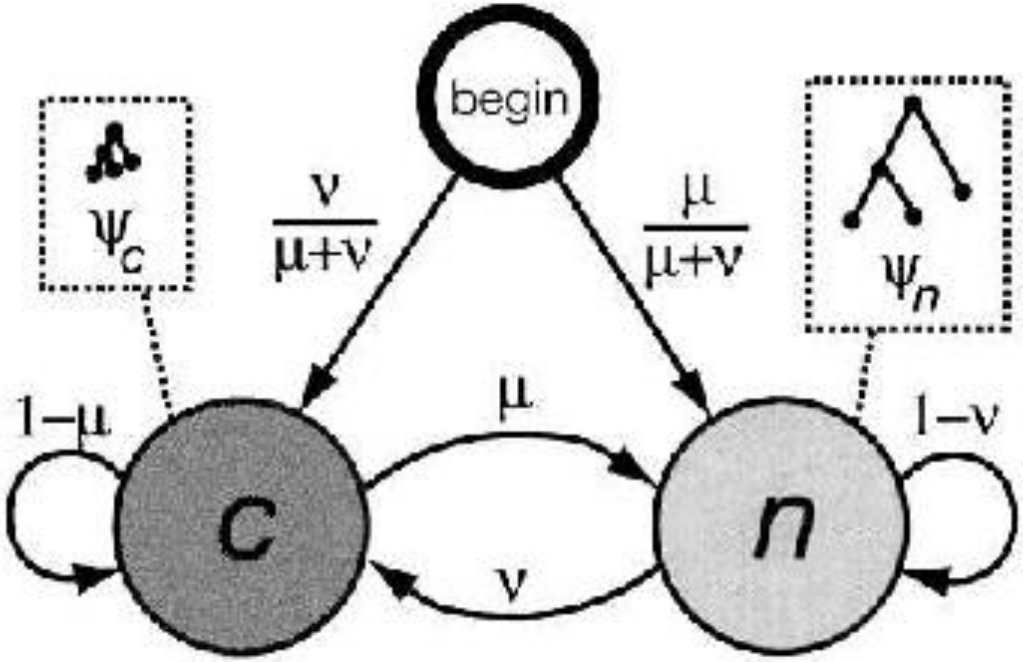
PhastCons PhyloHMM



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Some general issues in applying probability models, in the PhyloHMM context

- Is the model computable?
- Is the model ‘reasonable’?
  - 2 states enough?
    - variability of mutation, selection within genome
    - changes in selected sites over time
    - but simplicity has its advantages!
      - interpretability
      - overfitting & parameter estimation less problematic
  - Markov condition on transition probabilities
  - treatment of gaps

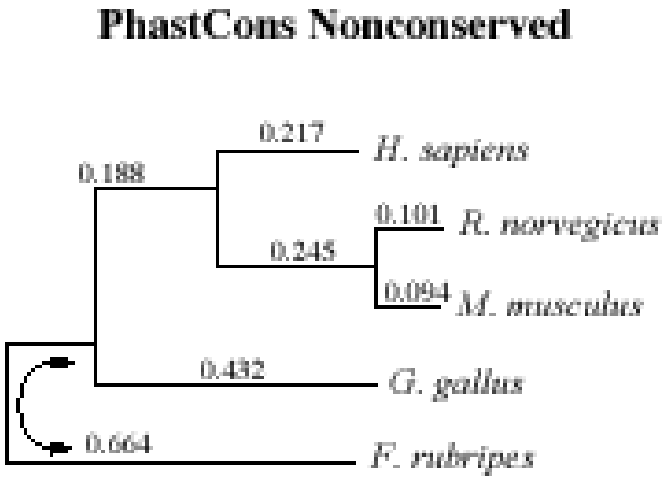
- How good is the input data?
  - alignability of neutral sequence
  - accuracy of genome sequence alignments

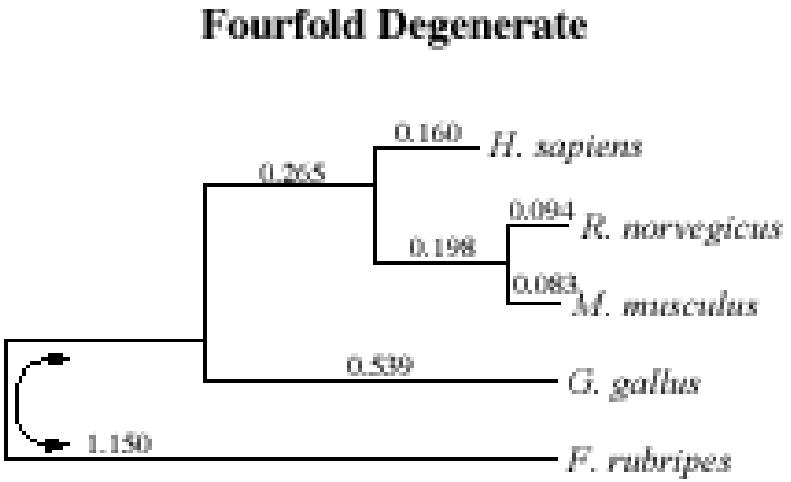
- Are results reliable?
  - no true ‘test set’ – instead, putative false positive rate, and ‘biological plausibility’ of findings

Alignment issues

- Multiz: progressive pairwise alignments
- accurate multiple genome alignment *not* a solved problem!
  - statistical assessment: Prakash & Tompa (2005, 2007, 2009)
  - ENCODE region alignment analyses: Margulies EH *et al.* 2007
  - major issues:
    - accurate gap placement (even for close species!!)
    - discrimination among paralogous sequences (e.g. repeats, duplications)
    - short 'junk' alignment segments
  - *in principle*, more sequences should give more accurate alignments
- inaccurate alignments can cause
  - neutral rate to be *overestimated*
  - conserved segments to be *overidentified*
    - because more slowly mutating (or better aligned) neutral segments may be called conserved

- for distantly related species, neutrally evolving regions no longer alignable
  - analyze 4D sites in coding sequences to estimate neutral rates
    - CDS alignments much more reliable, but
    - synonymous sites somewhat atypical (some selection; composition & mutation patterns)





The Genetic Code

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G