RAD MICROBES

BOOT CAMP ON READING, ASSEMBLING, ANALYZING & DECHIPHERING MICROBIAL GENOMES

APRIL 28-29, 2025 | BRC AT RICE UNIVERSITY | HOUSTON, TX

Phylogenetics

An Dinh

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Goals

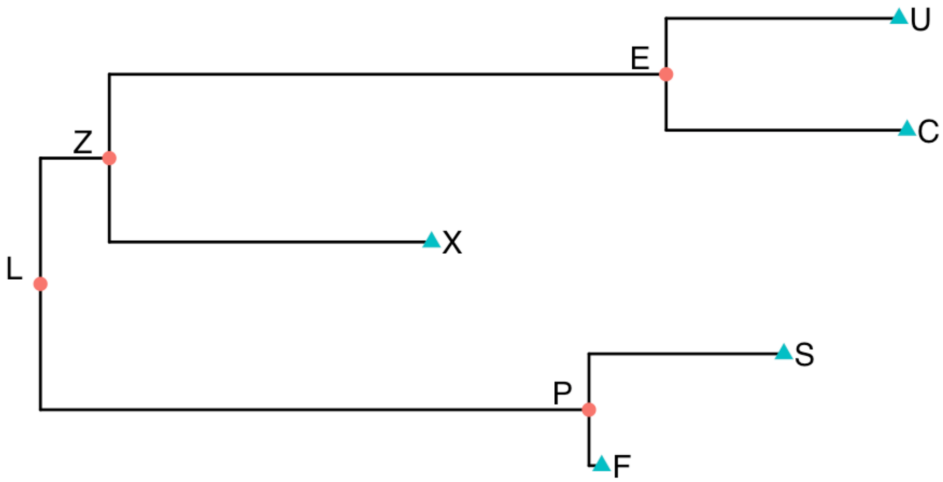
- Introduction to methods and models for phylogenetic inference
- Common language
- Demystification

Outline

- Tree basics
- Building a tree
- Distance matrix methods
- Character-based methods
  - Maximum parsimony
  - Maximum Likelihood
    - Heuristics
    - bootstrapping
  - Bayesian Inference
- Other complexities
- Takeaway

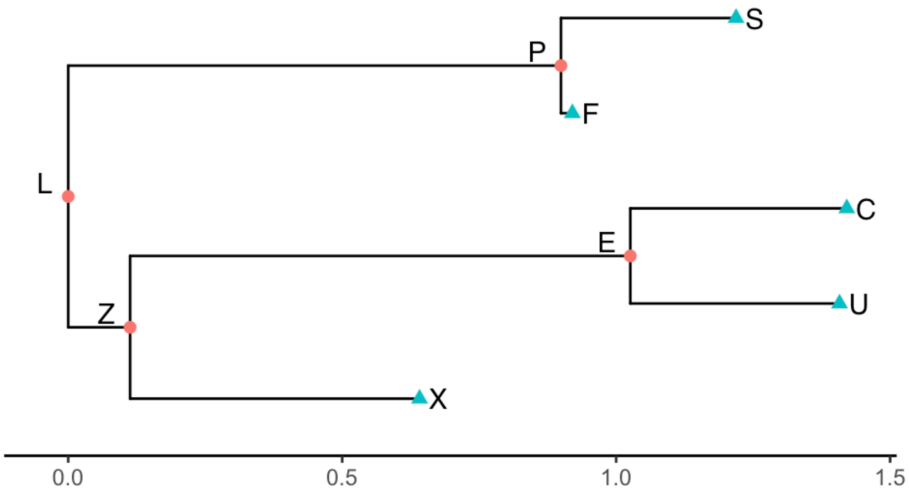
Dissecting a Tree

- Node (vertex) = divergence point
  - Internal nodes = nodes with at least one child branch
  - External nodes = nodes without child branches. Aka: “leaves”, “tips”,
- Branch (edge) = connection
  - Branch length represents the amount of distance
  - Can be external (terminating at a leaf), or internal



More on Phylogenetic Trees

- Observed (sequenced) samples are on branch tips.
- The order of the leaves don't really mean anything
- Most Recent Common Ancestor (MRCA
  - Or, Last Common Ancestor (LCA)
  - Located on internal node and Hypothetica



Unrooted vs. Rooted

- Unrooted shows evolutionary *relationships*

- Rooted shows evolutionary *history*
  - Allows for directionality
  - Time runs from root to tip
  - Root = MRCA of all the taxa in a tree

- Rooting a tree
  - **Outgroup** – common
  - Time-irreversible models (will cover briefly later)

Generalized workflow to build a tree

- **Data generation & QA/QC** – Session 1 & 2
- **Multiple Sequence Alignment** – Session 2 & 3
- **Tree estimation**
- Garbage in, garbage out!

Constructing Phylogenetic Trees

- Distance Matrix-based
  - Algorithmic
- Character-based
  - Estimate the optimum tree out of a set of trees

Distance matrix-based methods

- Start with an MSA, compute All-to-All distance matrix…

- Unweighted Pair-Group Method with Arithmetic Mean **(UPGMA)**
  - Mutational rate is uniform
  - Branch lengths are equally split
  - **Fitch-Margoliash (FM)** Method modifies this to do least-squares estimate of branch length

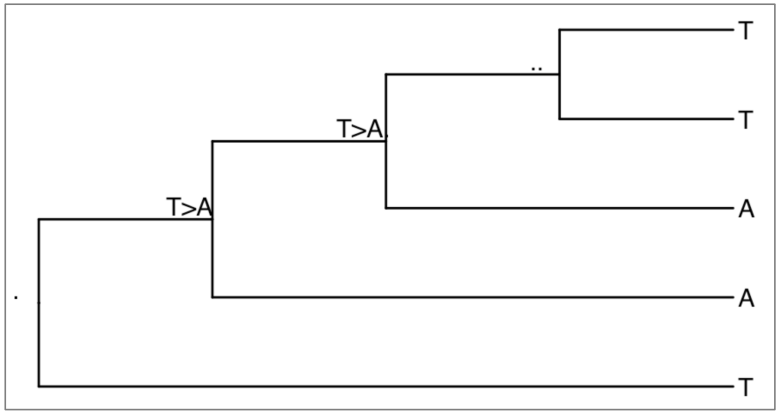
- **Neighbor-Joining (NJ)** - Saitou and Nei, 1987
  - Pairwise distance matrix → overall divergence → *adjusted* distance matrix
  - Not a bad heuristic and sometimes useful for initial starting tree

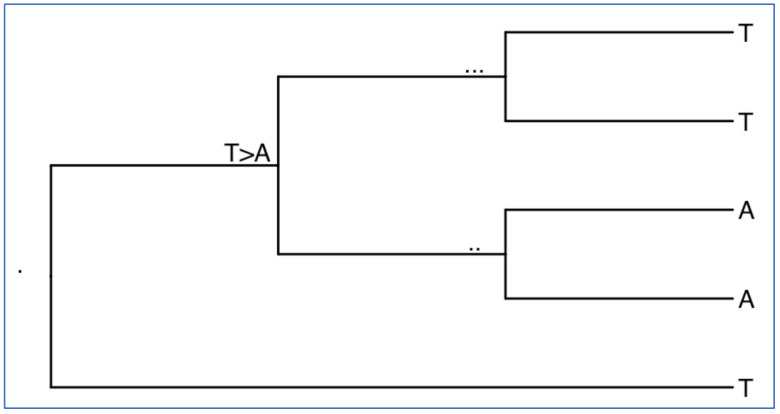
Constructing Phylogenetic Trees – **character-based**

- Maximum Parsimony
- Maximum Likelihood
- Bayesian Inference

Maximum Parsimony Positively misleading

- Out of all possible trees, the best tree will have the least number of evolutionary (substitution) events
- Assumptions
  - Homologous similarity – identical alleles came from a common ancestor
  - Homoplasy (such as convergent evolution or reversal) is rare





Maximum Likelihood

- **max(Pr(Data|Tree)),** given:
  - A sample set of sequences, and
  - A nucleotide substitution (evolutionary) model

- "Tree" is a model consisting of:
  - Topology (τ)
  - Branch lengths (ν)
  - Substitution model parameters (θ)

- Computationally intensive, will illustrate shortly…

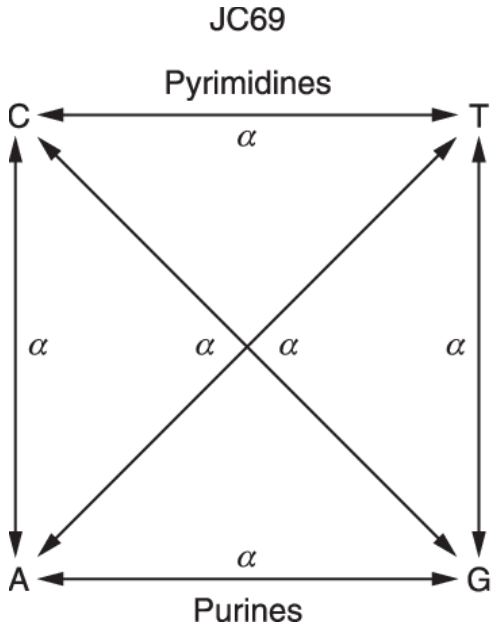
Nucleotide substitution models

Model	df	Explanation	Code
JC or JC69	0	Equal substitution rates and equal base frequencies (Jukes and Cantor, 1969).	000000
F81	3	Equal rates but unequal base freq. (Felsenstein, 1981).	000000
K80 or K2P	1	Unequal transition/transversion rates and equal base freq. (Kimura, 1980).	010010
HKY or HKY85	4	Unequal transition/transversion rates and unequal base freq. (Hasegawa, Kishino and Yano, 1985).	010010
TN or TN93	5	Like `HKY` but unequal purine/pyrimidine rates (Tamura and Nei, 1993).	010020
TNe	2	Like `TN` but equal base freq.	010020
K81 or K3P	2	Three substitution types model and equal base freq. (Kimura, 1981).	012210
K81u	5	Like `K81` but unequal base freq.	012210
TPM2	2	AC=AT, AG=CT, CG=GT and equal base freq.	010212
TPM2u	5	Like `TPM2` but unequal base freq.	010212
TPM3	2	AC=CG, AG=CT, AT=GT and equal base freq.	012012
TPM3u	5	Like `TPM3` but unequal base freq.	012012
TIM	6	Transition model, AC=GT, AT=CG and unequal base freq.	012230
TIMe	3	Like `TIM` but equal base freq.	012230
TIM2	6	AC=AT, CG=GT and unequal base freq.	010232
TIM2e	3	Like `TIM2` but equal base freq.	010232
TIM3	6	AC=CG, AT=GT and unequal base freq.	012032
TIM3e	3	Like `TIM3` but equal base freq.	012032
TVM	7	Transversion model, AG=CT and unequal base freq.	012314
TVMe	4	Like `TVM` but equal base freq.	012314
SYM	5	Symmetric model with unequal rates but equal base freq. (Zharkikh, 1994).	012345
GTR	8	General time reversible model with unequal rates and unequal base freq. (Tavare, 1986).	012345

Basic Markov chain models for substitution

- **Jukes-Cantor** (1969)
  - Rate of nucleotide substitution is the same for all pairs

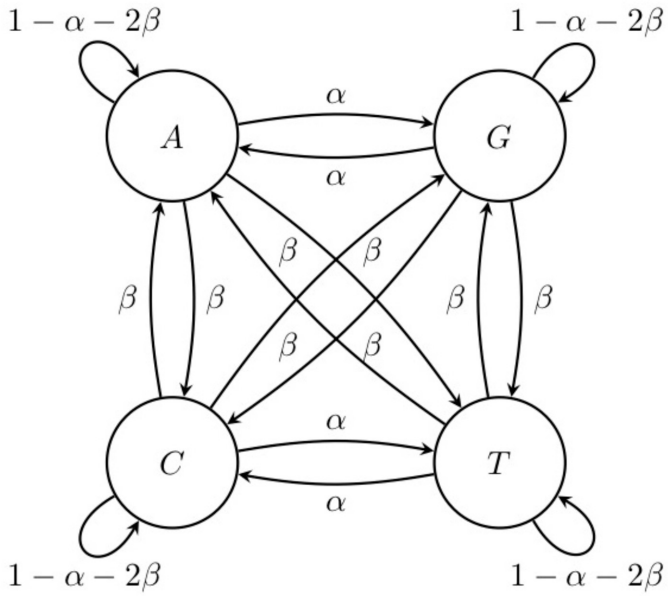
- See also: **Felsenstein** (1981)
  - Like Jukes-Cantor (equal substitution rates), but with unequal base frequency



Egan, Ashley & Crandall, Keith. (2006). Theory of Phylogenetic Estimation. 10.1093/oso/9780195168174.003.0028.

More basic models for substitution

- **Kimura** (1980)
  - Transitions (intragroup changes) have a different rate than Transversions (intergroup changes



- See also: **Hasegawa-Kishino-Yano** (1985)
  - Like Kimura (unequal transition vs. transversion rates), but with unequal base frequency

More complex (less constrained) substitution models - GTR

- **General Time-Reversible** substitution model - (Tavare 1986)
  - Like HKY, but each possible nucleotide pair has it's own "exchangeability rate" along with unequal base frequencies
  - "Generalised time reversible (GTR) is the most general neutral, independent, finite-sites, time-reversible model possible."

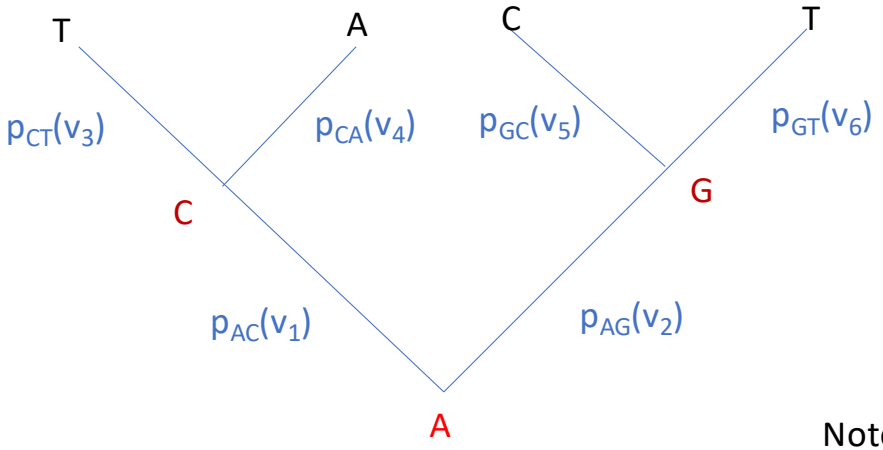
$$Q = \begin{pmatrix} -(a\pi_C + b\pi_G + c\pi_T) & a\pi_C & b\pi_G & c\pi_T \\ a\pi_A & -(a\pi_A + d\pi_G + e\pi_T) & d\pi_G & e\pi_T \\ b\pi_A & d\pi_C & -(b\pi_A + d\pi_C + f\pi_T) & f\pi_T \\ c\pi_A & e\pi_C & f\pi_G & -(c\pi_A + e\pi_C + f\pi_G) \end{pmatrix}$$

Model modifications

- **+Γ** (Yang 1994)
  - Removes the assumption of per-site independence
  - **Models rate-heterogeneity** in mutational rates across sites (ie: not all sites have equivalent nor independent mutational rates)
  - Applies gamma distribution with a shape parameter (alpha), itself usually estimated from the sample data
- **+ I** (invariant sites)

Computing the likelihood

- Given a possible tree topology…
  - At a *single* site i…
    - Assume we "know" interior nodes; leaves are observed



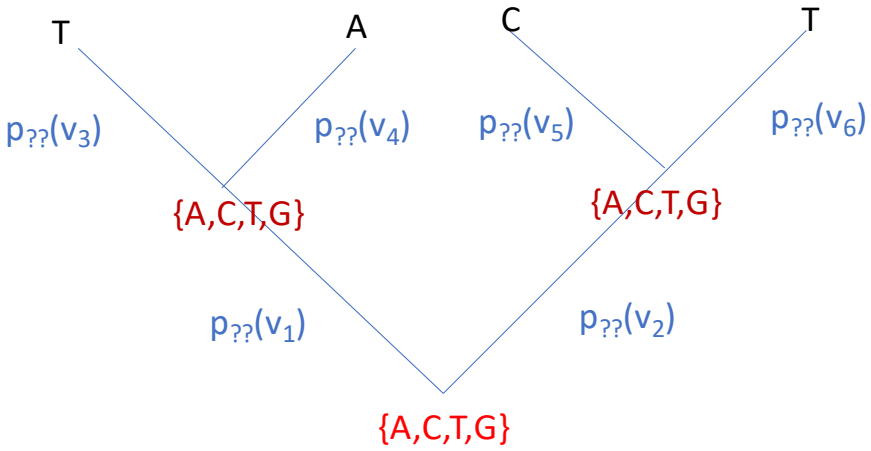
Multiply

$$L(T|D) = \Pr(D|T) = \pi_A \cdot p_{AC}\ (v_1) \cdot p_{AG}\ (v_2) \cdot p_{CT}\ (v_3) \cdot p_{CA}\ (v_4) \cdot p_{GC}\ (v_5) \cdot p_{GT}\ (v_6)$$

Note: we don't actually know what the branch lengths are yet,
And we may not have substitution parameters estimated yet either…

Computing the likelihood (2)

- Given a possible tree topology…
  - At a *single* site i…
    - BUT we do NOT know what the interior nodes are… they could be anything.



$$L(T|D) = \sum\sum\sum L(all\ possible\ inner\ node\ assignments)$$

Sum over

$$4\times4\times4 = 64\ combinations$$

**Fortunately, Felsenstein's Pruning Algorithm brings complexity down to $O(nsc^2)$

But then consider…

- Number of sites

- How many possible trees:
  - $N_{unrooted} = (2n - 5)!!$
  - $N_{rooted} = (2n - 3)!!$

  - Let n = 10 sequences
    - $N_{unrooted} = (2n - 5)!! = (15)!! = 15 * 13 * 11 * 9 * 7 * 5 * 3 * 1 = 2027025$
    - $N_{rooted} = 3.45\text{e}7$

- Exhaustive search infeasible; ML criterion is *NP-hard*

Heuristic (uphill) search algorithms for tractability

- Nearest-neighbor interchange (NNI)
  - Given a topology, local arrangements are assessed for likelihood by successively deleting interior branches and testing the other possible reconstructions
  - Needs branch length (sequence distances) to compute
- Subtree prune and regraft (SPR) – **RAxML**
- Tree bisection and reconnection (TBR)

Bootstrapping for Branch Support and reliability

- In general, bootstrapping = random subsampling *with replacement* (resampling)
- Estimates the statistical error when sampling distribution is unknown

- In phylogenetic context, estimates the reliability (consistency) of the resultant (majority-rule) **consensus tree**
- Bootstrapped sites will be randomly shuffled, length same as original MSA.

- The final resultant tree might not be the overall maximum-likelihood tree

Bayesian Inferential Methods

- $\Pr(\boldsymbol{Tree}|\boldsymbol{Data}) = \frac{\boldsymbol{Pr}(\boldsymbol{Data}|\boldsymbol{Tree})\cdot\boldsymbol{P}(\boldsymbol{Tree})}{\boldsymbol{P}(\boldsymbol{Data})}$
  - $Pr(D|T)$ = likelihood, but not the same as in ML method due to handling of 'nuisance parameters'
  - $\Pr(T)$ = prior probability distribution: typically assume uniform initially, ie: all trees are equally likely
  - $Pr(D)$ = a normalizing constant, $= \sum(P(D|T)\ x\ P(T_i))$

- Can sometimes be faster than ML but convergence is never certain

Bayesian Inferential Methods

- Uses Markov Chain Monte Carlo (MCMC) search methods
  - Metropolis coupled MCMC
  - Stochastic algorithms which should (in theory) help avoid getting trapped in sub-optimal solutions

  - Start with a random tree
  - Attempt a random walk
    - If the next tree is better than the one you're at, move there.
    - If the next tree is not better, then *maybe* move there, depending on how much worse it is.

More complexities to address mutational heterogeneity

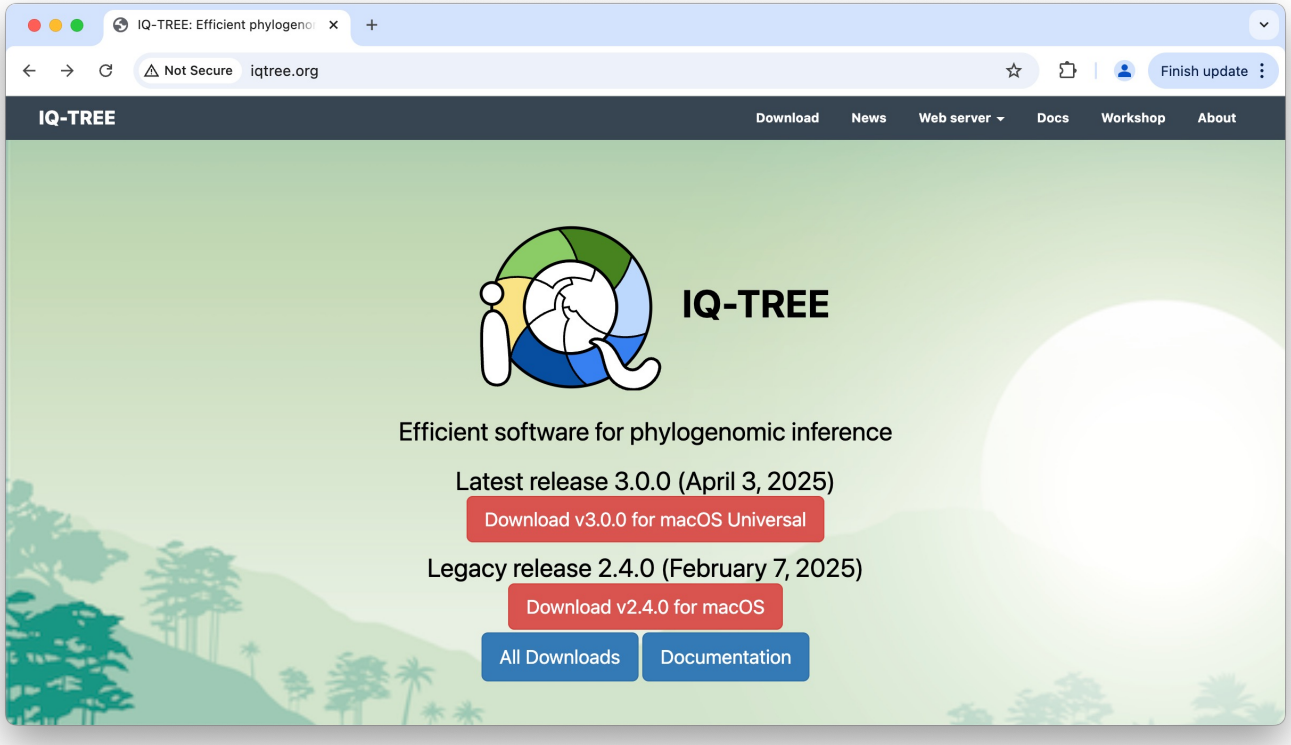
- **Partitioning** (categorization)
  - Apply specific models for a site or regions along a sequence
- **Mixture** models
  - Assign a model (out of a user-specified selection) on a per-site basis
- Some caution may need to be exercised to avoid over-parameterization

Non-reversible models

- **UNREST** (Yang 1994)
  - Completely unrestrained
- "Rootstrap" = bootstrap + root position support
- Relaxes the constant-rate assumption for molecular clock modelling

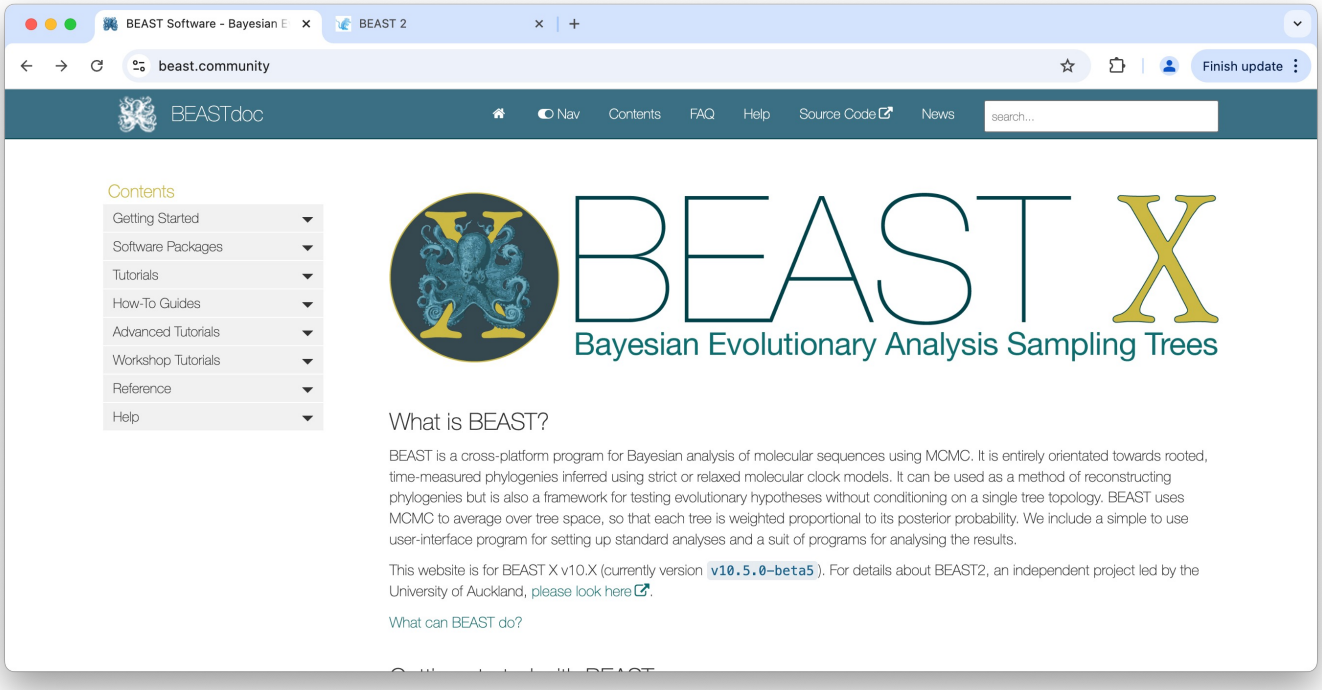
$$\mathbf{Q} = \begin{bmatrix} \cdot & q_{TC} & q_{TA} & q_{TG} \\ q_{CT} & \cdot & q_{CA} & q_{CG} \\ q_{AT} & q_{AC} & \cdot & q_{AG} \\ q_{GT} & q_{GC} & q_{GA} & \cdot \end{bmatrix}$$

IQ-TREE



- "combine elements of:
  - hill-climbing algorithms (NNI)
  - random perturbation of current best (locally optimal) trees,
  - and a broad sampling of initial starting trees"
- Ultrafast bootstrapping
- Feature rich
- Good Documentation

BEAST1/BEASTX ; BEAST2



- BEAST2 more modular, packages include MSC

Takeaways

- Balancing between robustness and efficiency
  - Computational time, memory consumption…
- Inferential methods (ML, Bayesian) are statistically consistent
- GTR is probably the most popular model
- At least some form of rate heterogeneity modelling should be employed

Evolutionary processes leading to non-tree-like structures and Phylogenetic conflicts

- Horizontal Gene Transfer
  - Transformation
  - Conjugation
  - Transduction
  - Gene transfer agents (ie: defective prophages)
- Gene Duplication and Loss
- Transposition of Mobile Genetic Elements

- What now???
  - Networks?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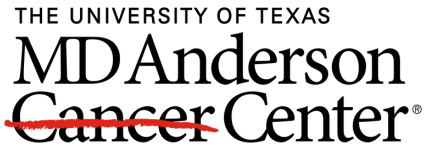
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