What Is Phylogenetic/Phylogenomic Analysis?

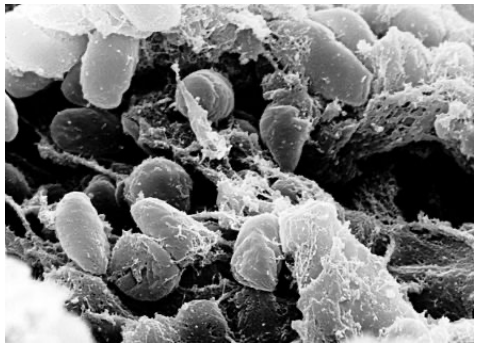
Phylogeny = Evolutionary history

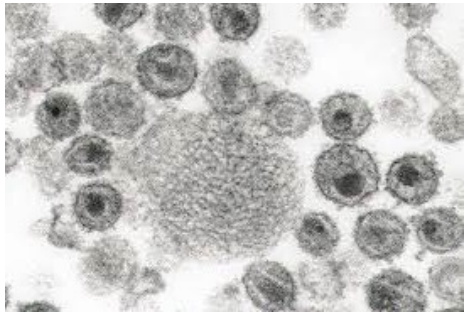
Reconstruction of evolutionary history using shared traits among organisms.

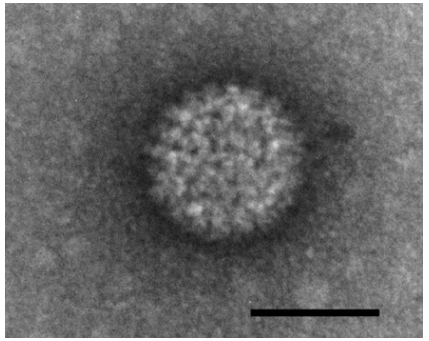
- Tree of life *http://www.tolweb.org/tree/*
- Time Tree *http://www.timetree.org*

DNA Sequencing of Pathogens

- Genome organization, structure and function
- Estimation of pattern and rate of mutation within each pathogen gene
- Estimation of genetic diversity within the pathogen
- Identification of emergent strain
- Geographic and evolutionary origin of emergent strain(s)
- Patterns of global transmission
- Co-evolution and adaptation
- Selection
- Fitness traits-replication rate, transmissibility, immune recognition
- Intra-host diversification and horizontal gene transfer among strains
- Vaccine and drug therapy development







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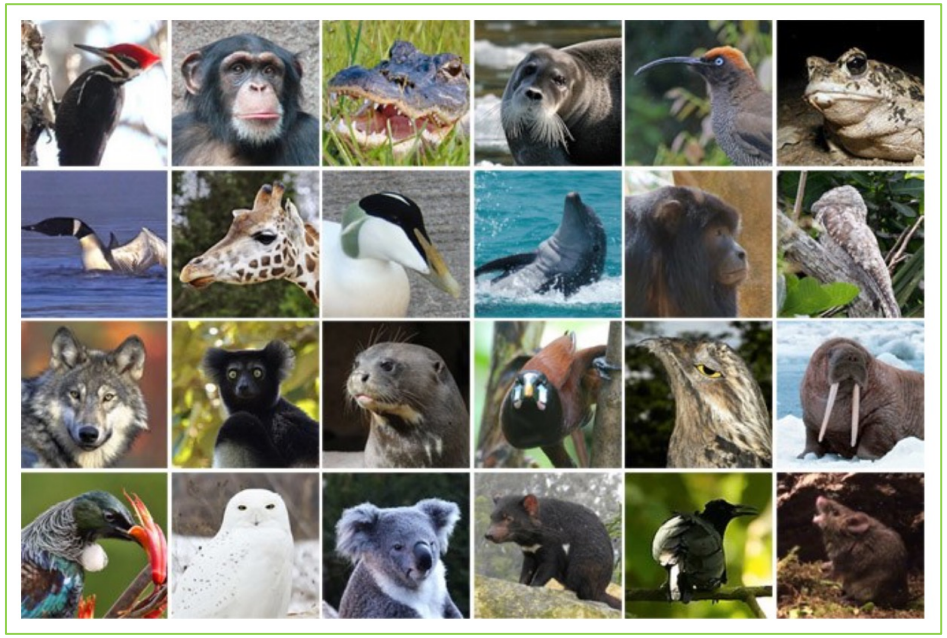
DNA Sequencing of Host Species

- Genome structure, content, function, evolution
- Evolutionary history of species
- Population structure and phylogeography
- Historic and ongoing patterns of migration
- Genetic diversity
- Inbreeding
- Domestication
- Hybridization
- Endangered or relic species, subspecies and populations
- Identification of genes involved in disease resistance and progression
- Predictive effects of pathogen emergence in naïve host populations



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Phylogenetic Tree: Visualizations

- Phylogram
  - Tree branch length proportional to genetic divergence
- Cladogram
  - Topology only, branching order important
  - Branch lengths ignored
  - Character data
- Rooted
  - Outgroup
  - Midpoint rooting
- Unrooted

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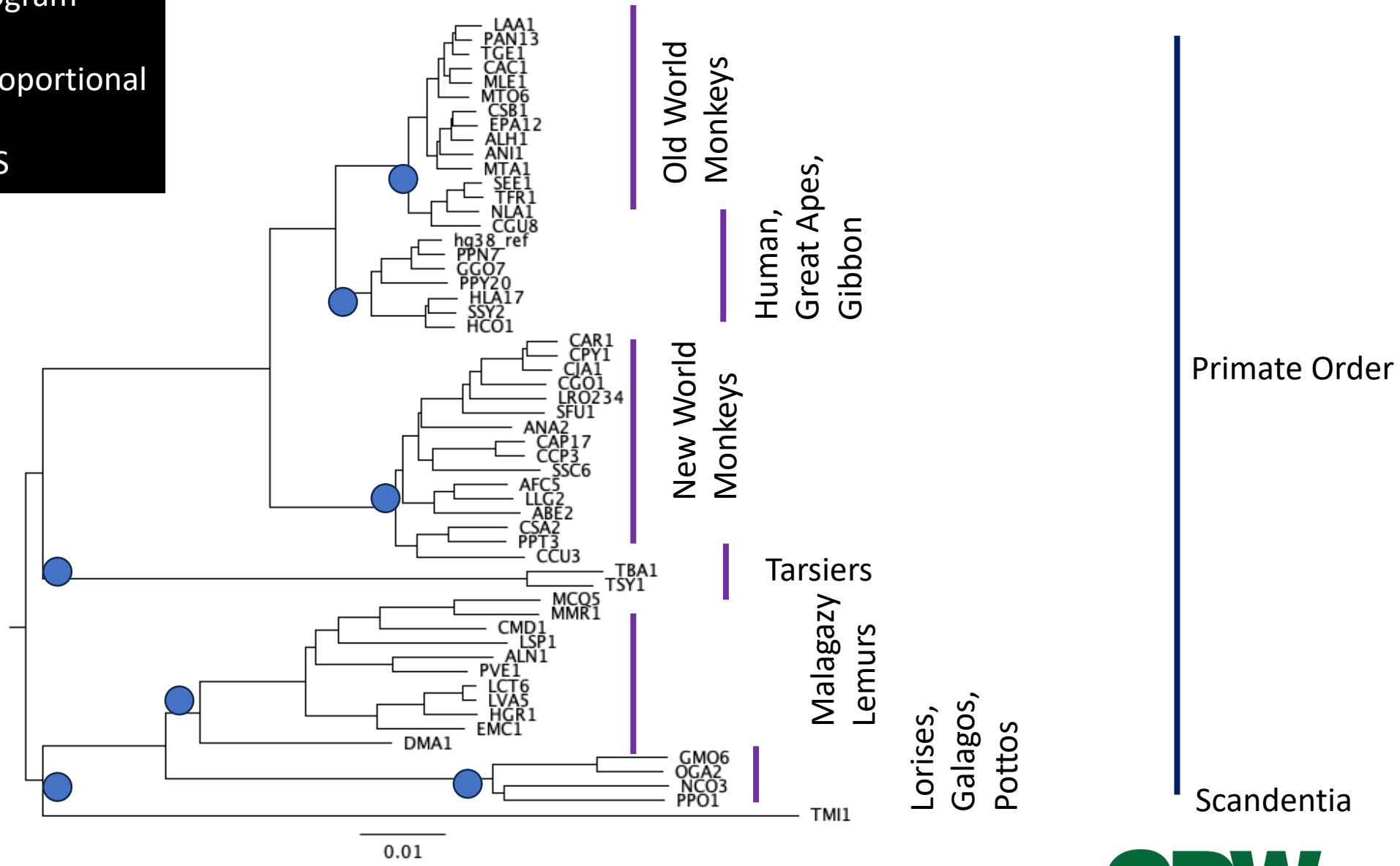
Rectangular Phylogram

Branch lengths Proportional

All nodes 100% BS

Primate Genera Tree Based on 10,000 genes



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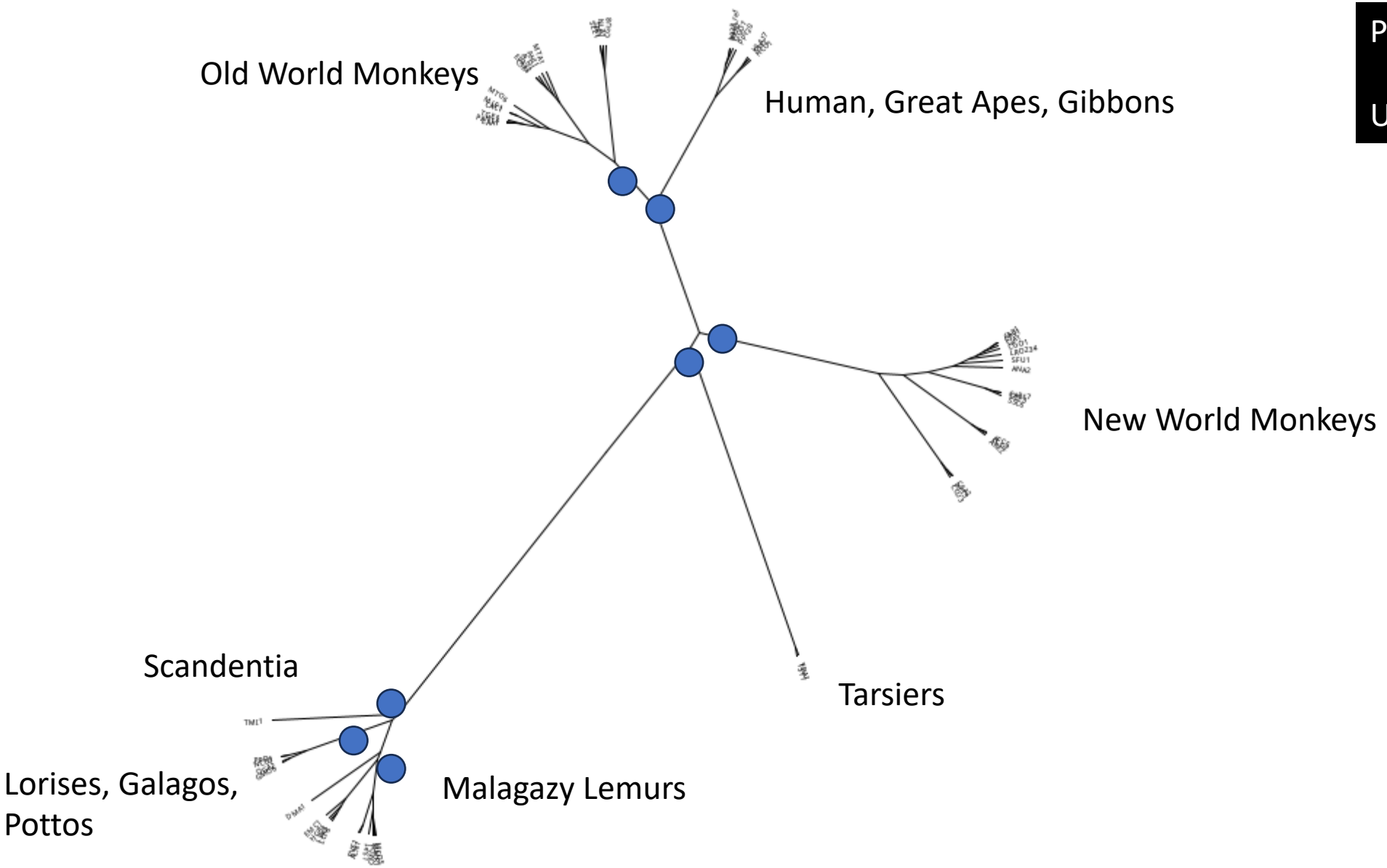
Primate Order

Unrooted Phylogram



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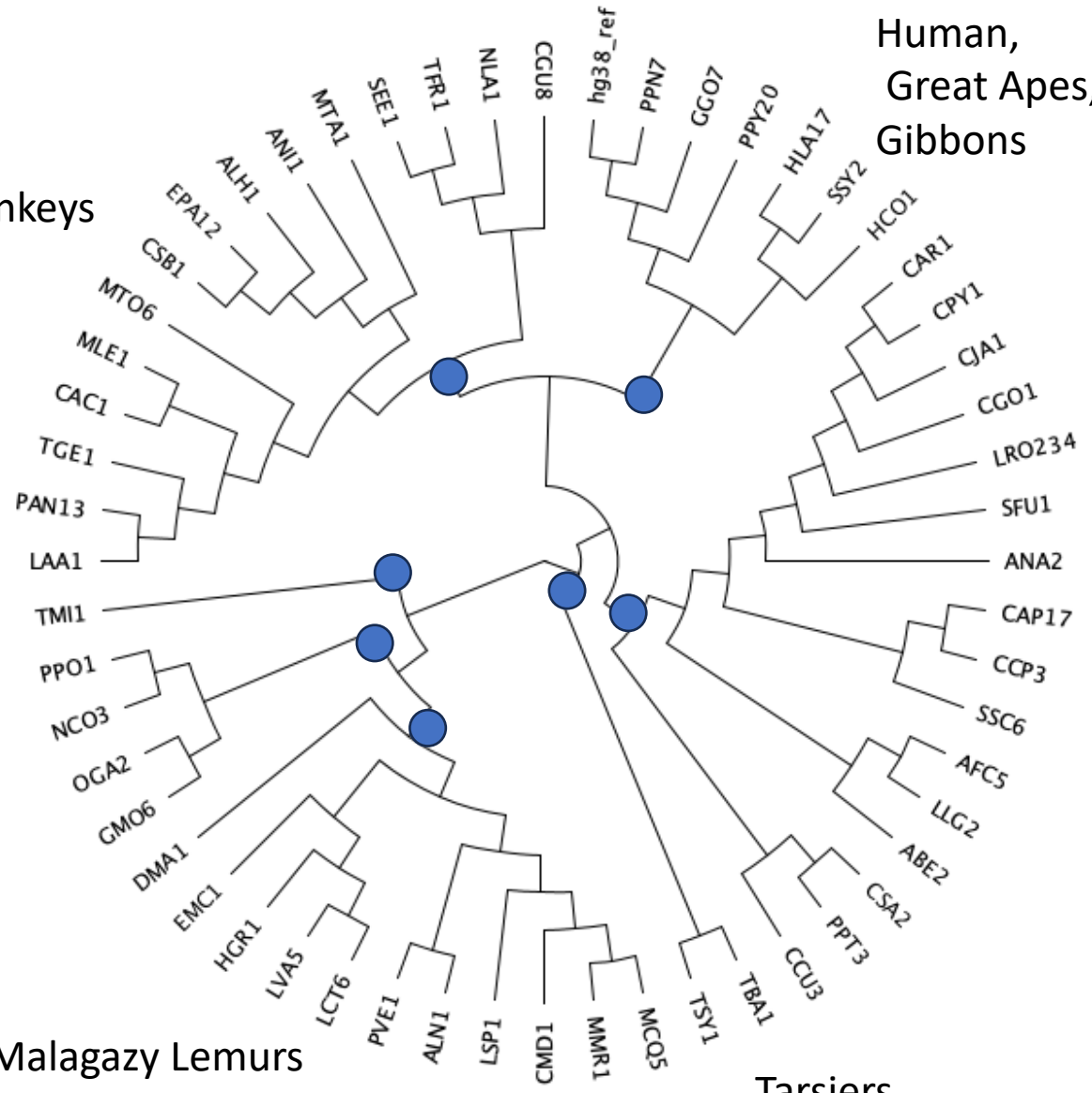
Circular Cladogram:

Branching order only
Branch lengths ignored



Human,
Great Apes,
Gibbons

Old World Monkeys

Scandentia

Lorises, Galagos, Pottos

New World Monkeys

Malagazy Lemurs

Tarsiers

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Elements for Setting Up the Analyses

Step 1: Determination of informative marker(s) for Hypothesis

Step 2: Determination of sample size and design

- Taxa or OTUs
- Outgroup-root

Step 3: Alignment (sequence data)

Step 4: Model of substitution

- Nucleotide data
  - Among site rate heterogeneity
  - Bias frequency nucleotides
  - Transition: Transversion
  - Codon analyses
- Protein Data
  - Probability matrix [DAYHOFF, DCMUT, JTT, MTREV, WAG, RTREV, CPREV, VT, BLOSUM62, MTMAM, LG] or empirical frequencies of amino acid within data set.

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Building and Assessing the Phylogeny

Step 5. Select optimality criteria and create phylogeny

- Maximum Likelihood (probability scores for observed variation)
- Minimum Evolution (distance-based)
- Maximum Parsimony (character states)
- Bayesian Inference (MCMC)

- Computer Programs
  - IQTree, PAUP, MEGA, RAxML, PhyML, MrBayes

Step 6. *A posteriori* evaluation

- Bootstrap
  - Assessment of tree reliability
  - Re-sampling with replacement
  - Node support
- Jackknife
  - Random sampling without replacement
  - Sites or taxa
- Bayesian Posterior Probability of Model Parameters

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Phylogenetic Data Input

Whole Genome Comparisons

- Comparison of syntenic regions (Conserved Sequence Blocks)

Protein Coding Data (Genes and Exomes)

- Homologs versus Paralogs
  - Gene duplication and Gene Loss
- Amino Acid versus Nucleotide
  - Synonymous substitutions
  - Nonsynonymous substitutions (missense mutations)

MtDNA

- Pattern of Inheritance Bias (Matrillineal)
- Single Evolutionary Unit despite different genes evolving at different rates

Autosomes, X & Y Chromosomes

- Y/X > 1 Male-driven evolution linked with spermatogenesis

Retro-elements

- SINEs, LINEs, Endogenous retroviruses

Microsatellites, SNPs, SNVs

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Data Selection: Hypothesis Driven

Informative: "Garbage In, Garbage Out"

Neutral Evolution: **Does it exist?**

First proposed by Kimura (1968): amino acid changes are selectively neutral driven by mutation rate and genetic drift.

Short answer : unlikely

Genomes and genes under strong selection

Maintain function (purifying selection)

Diversification to adapt or to gain function (positive selection)

Is this really a problem?

Quality of Data:

Robustness: Additional data strengthens overall topology

Accuracy: Data capture 'true' evolutionary and natural history

Precision: Data track similarly throughout phylogeny

Empirical Consensus: Combined Data and/or Multi-Gene Coalescence Methods

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Heuristic Searches To Find the Optimal Solution

Optimum:
Tree with the highest score possible under the different optimality criteria (ML, MP, ME, BAYESIAN)

Problem- Cannot test all possible combinations of taxa.
'Exhaustive search' -not recommended for more than 10 sequences in an alignment
e.g. an alignment of 20 sequences will require testing differences between 2×10^{20} potential trees

Solution: Heuristic searches designed as short cuts through all possible tree space:

Nearest neighbor interchange (NNI)
Subtree-Pruning-regrafting (SBR)
Tree bisection reconnection (TBR)

If suspect getting stuck local optimum:
rearrange input order, star decomposition, iterative step-wise addition.

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Phylogenetic Pitfalls

- Recombination
- Gene conversion
- Gene duplication
- Gene loss
- Selection
- Insufficient elapsed time since last shared common ancestor
- Ancestral polymorphisms
- Incomplete lineage sorting (discordant gene trees)
- Saturation of sites (multiple hits)
- Long branch attraction (inadequate taxon sampling)
- Inadequate models of amino acid or nucleotide substitution

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Lab: Phylogenomics with IQ-Tree: Ver 2.2.0

- **Accuracy**: Proposing novel computational methods that perform better than existing approaches.
- **Speed**: Allowing fast analysis on big data sets and utilizing high performance computing platforms.
- **Flexibility**: Facilitating the inclusion of new (phylogenomic) models and sequence data types.
- **Versatility**: Implementing a broad range of commonly-used maximum likelihood analyses.

IQ-TREE has been developed since 2011 and freely available at http://www.iqtree. org/ as open-source software

Bui Quang Minh, Rob Lanfear, Nhan Ly-Trong Jana Trifinopoulos, Dominik Schrempf, Heiko A. Schmidt March 25, 2022

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Tree-Based Inferences About Gene Function: An Real-World Example

- Hypothesis: What genes are evolving the fastest across the primate order?
  - All the same?
  - All the same among lineages?

- We came up with the top five list for nucleotides and amino acid data
  - Any guesses to what we saw?

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The aye-aye

Daubentonia madagascarensis

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Nucleotide Substitution Models

- Range from simple to complex
- Models are tested hierarchically, adding more complex elements
- Performs a likelihood test between successive models
- Selects a model based on AIC or BIC criteria

- **Lab:** Download IQRuns from GDW2023 Github to Desktop
- We'll test for a Model
- We'll find a Maximum Likelihood Tree
- We'll do a bootstrap

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