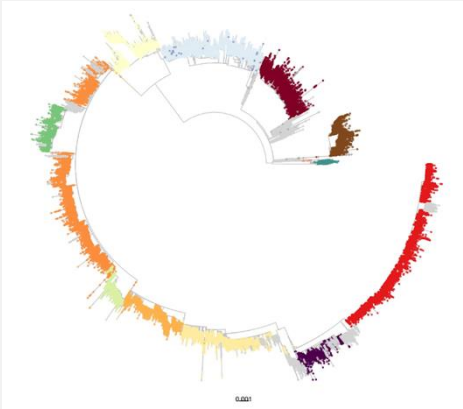
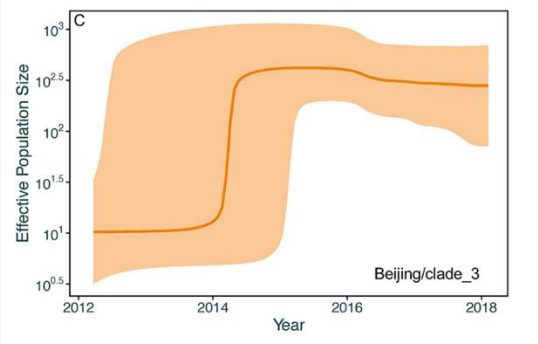
GENOMIC ANALYSIS AND PHYLODYNAMICS

Lecture 2: Variant Calling and Phylogenetic Trees





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MRC Senior Research Fellow, University College London

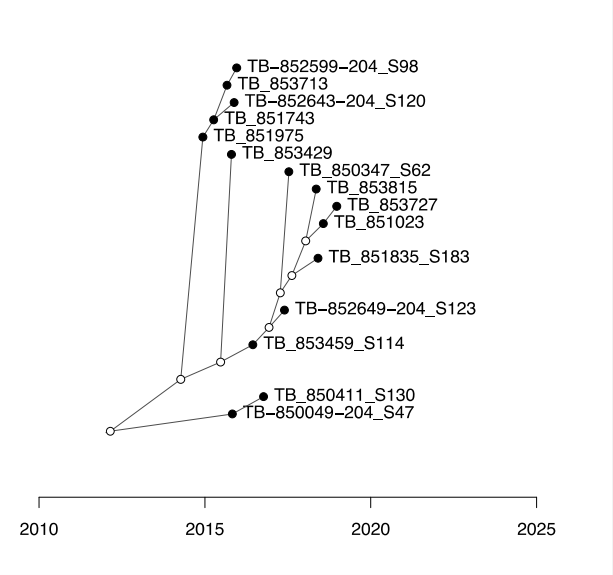
LECTURE 2: VARIANT CALLING AND PHYLOGENETIC TREES

- What can variation within the genome tell us?
- What are genomic variants?
- How do we detect variation in the genome?
- Linking variation to evolutionary relationships using phylogenetic trees

LECTURE 2: VARIANT CALLING AND PHYLOGENETIC TREES

What can variation in the genome tell us?

- Insights into the evolution of organisms and selection
  - Variations in the genome contribute to the adaptation of species to their environment
- Specific mutations may alter the protein that is coded for by a gene and change characteristics
- Analysing the amount and patterns of differences can relate to the amount of divergence and common ancestry between individuals
  - Can be linked to evolutionary history and transmission



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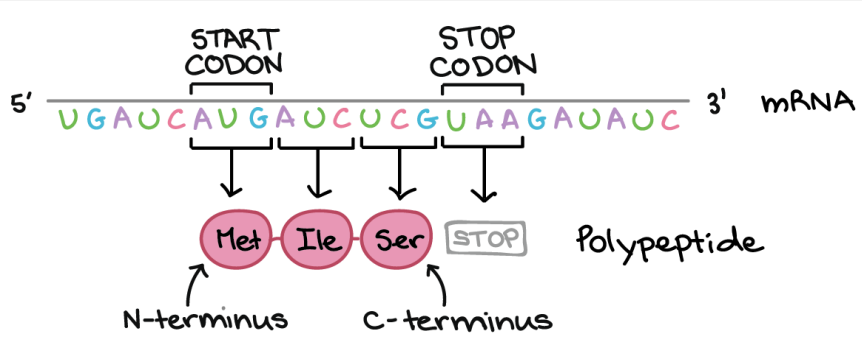
How the genome is read

- Process of reading the genome is transcription
- RNA polymerase binds to promotors (upstream of a gene) and then 'reads' the genetic code to make a complimentary RNA strand
- This RNA strand is then translated into proteins by building amino acid blocks coded for by the sequence
- A specific sequence of three nucleotide bases (codon) encodes an amino acid



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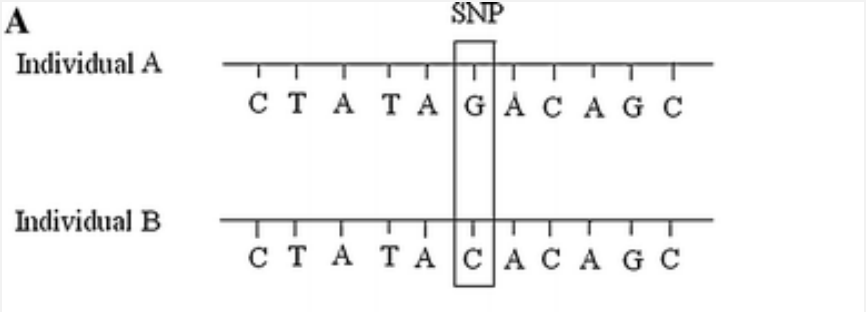
Single nucleotide polymorphisms

- Single Nucleotide Polymorphisms, or SNPs, are the most common type of genetic variation
- A point mutation where a single nucleotide base (A, C, G, or T) in the DNA sequence is replaced by one of the other three bases at the same position
- Most common form of genomic variation



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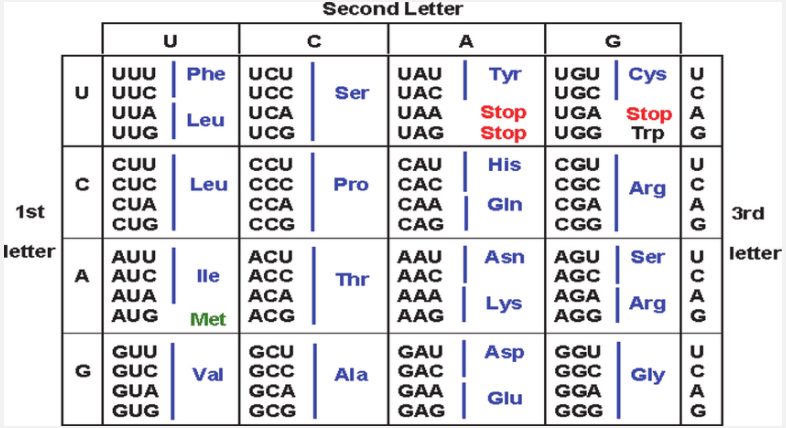
Single nucleotide polymorphisms

- SNPs can result in a change in the amino acid that is encoded (non-synonymous) or still code for the same amino acid (synonymous)
- There are 64 codons but only 20 amino acids (+ start and stop codons)



1st letter	Second Letter: U	Second Letter: C	Second Letter: A	Second Letter: G	3rd letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

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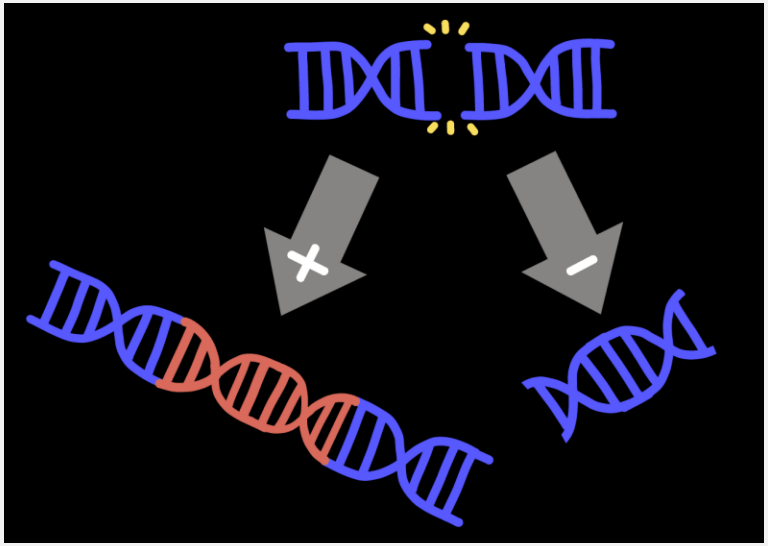
Insertions and deletions (Indels)

- Often abbreviated as Indels
- The addition or removal of one or more nucleotide bases
- Can have significant effects on the structure and function of genes and can contribute to genetic diversity within populations
- Can have most impact when they are 'frameshift' indels – changes the codon position



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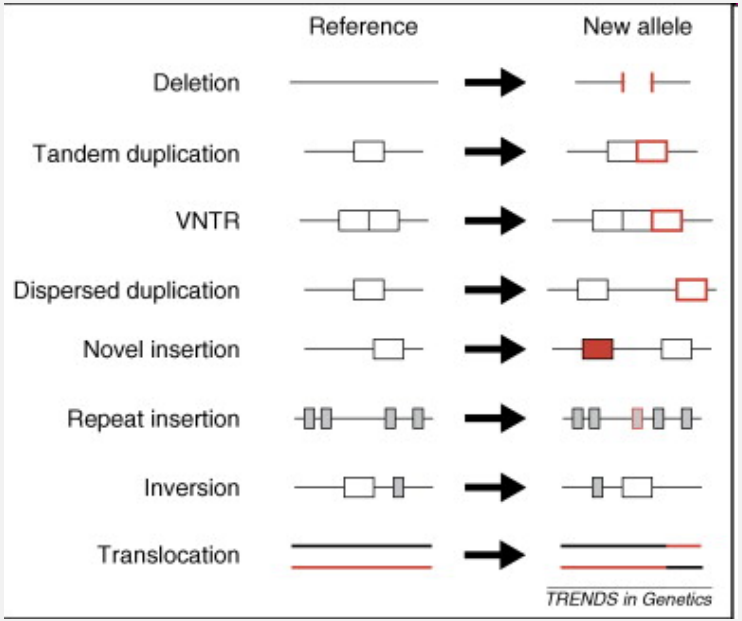
Other genomic variants

- Gene duplications
- Inversions
- Translocations
- Rarer, and more difficult to detect and analyze



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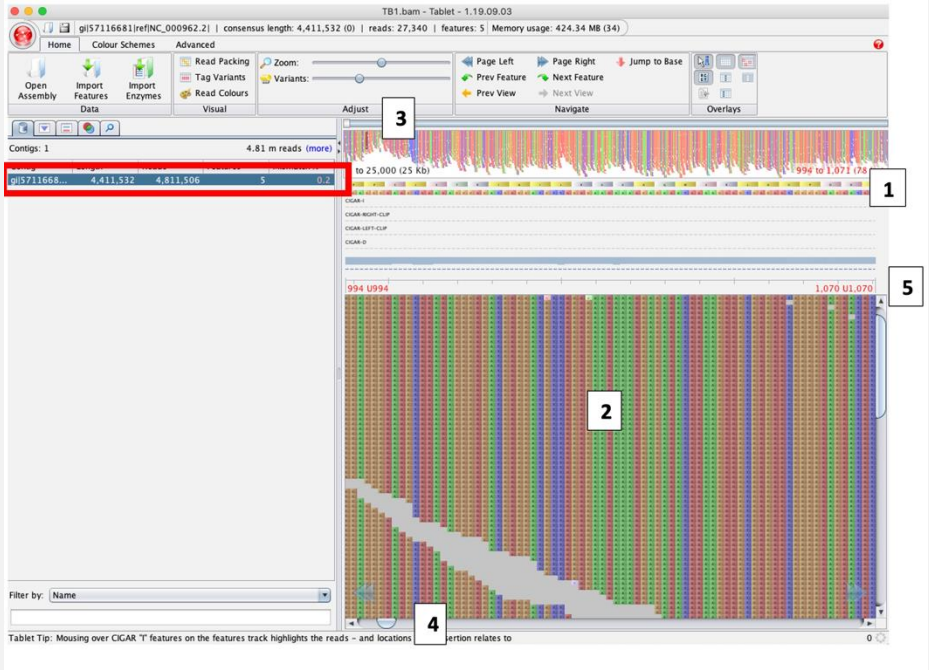
Assembled/aligned genomes

- We now have our assembled or aligned genome
- The file format is called a BAM (or SAM) file
  - A TAB-delimited text format consisting of an optional header and an alignment.
- Minimum format agreed on to report sequencing results, and includes all the data in a *fastq* file



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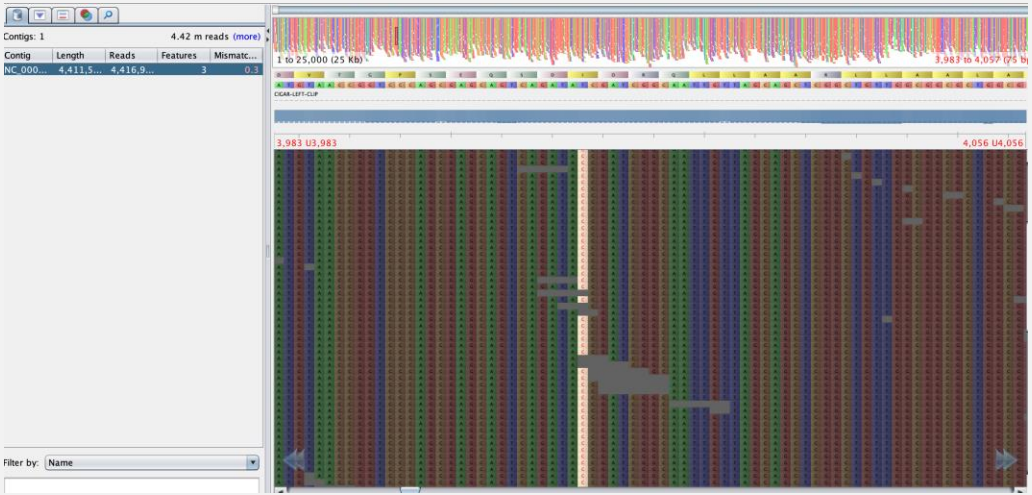
Assembled/aligned genomes

- Could scroll through the alignment file to detect variation
- But, time-consuming and subjective – how do we decide between variation and error?
- We can use tools to read through the BAM file and identify true variation



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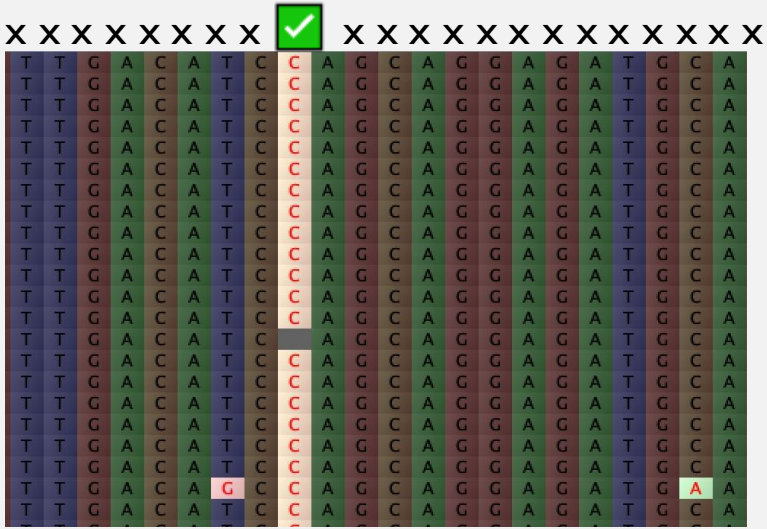
Identifying variants

- We use modelling approaches to identify variants between the reference and sequences of samples, and between samples.
- Need to maximize sensitivity/specificity of calls by accounting for sequence error, poor alignment, low quality etc.



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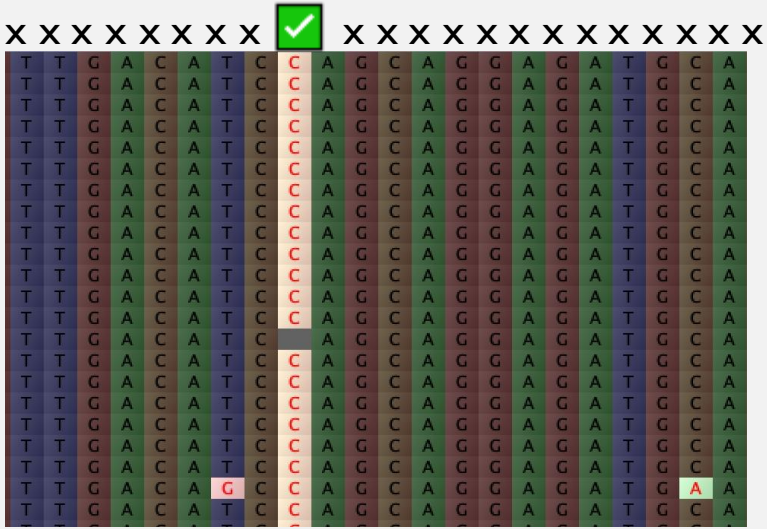
Variant calling software

- Multiple variant calling software (for short-read data) are available including:
  - GATK
  - SAM/BCFtools
  - Delly
  - Pilon
  - FreeBayes
  - DeepVariant
- Have different strengths depending on the type of variants to call



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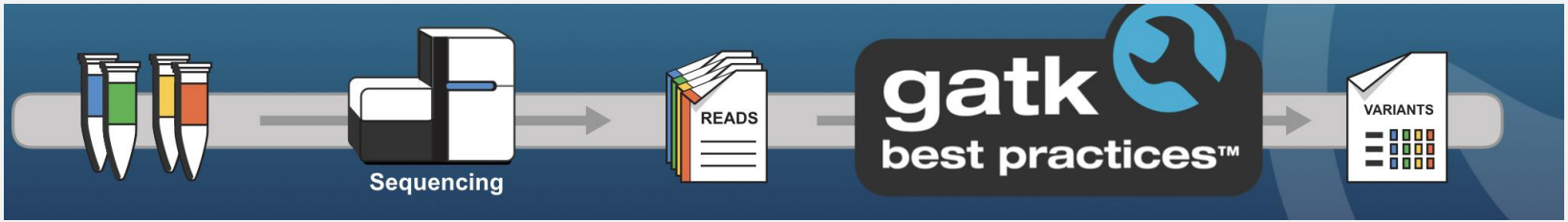
GATK variant calling

BAM file(s) -> Machine learning model to recalibrate base quality scores -> Bayesian genotype likelihood model to call variants at each genomic position -> Filters variant calls to reduce false positives -> Quality Control and Validation (manual)



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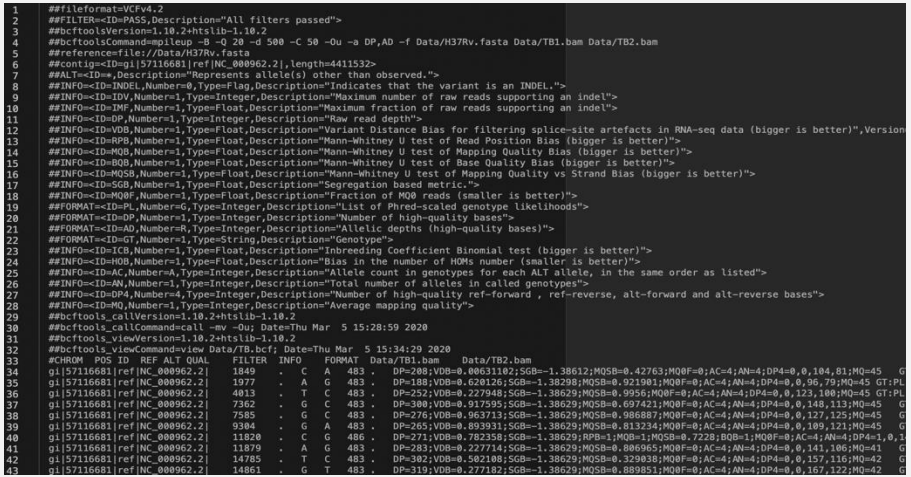
Variant Call Format (VCF) files

- The Variant Call Format (VCF) is a widely used file format in genomics for storing information about genetic variants, such as single nucleotide polymorphisms (SNPs), insertions, deletions, and other types of genetic variation.
- Important part is the Genotype data - the alleles carried by each individual at the variant site.
  - e.g., "0/0" for homozygous reference, "0/1" for heterozygous, "1/1" for homozygous alternate



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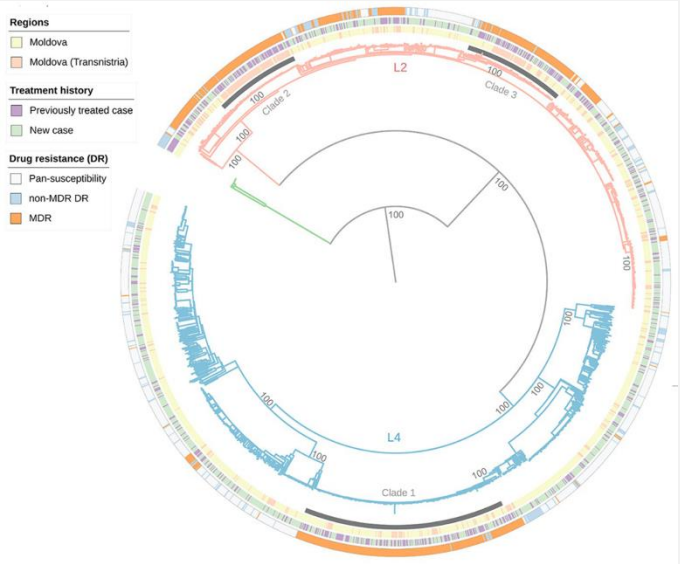
LECTURE 2: VARIANT CALLING AND PHYLOGENETIC TREES

Phylogenetic trees

- A phylogenetic tree is a diagram that represents evolutionary relationships among organisms
- They illustrate the ancestral lineage and divergence of species, genes, or other taxonomic units.
- These trees help in understanding evolutionary history, inferring patterns of descent, and clarifying the timing of evolutionary events.



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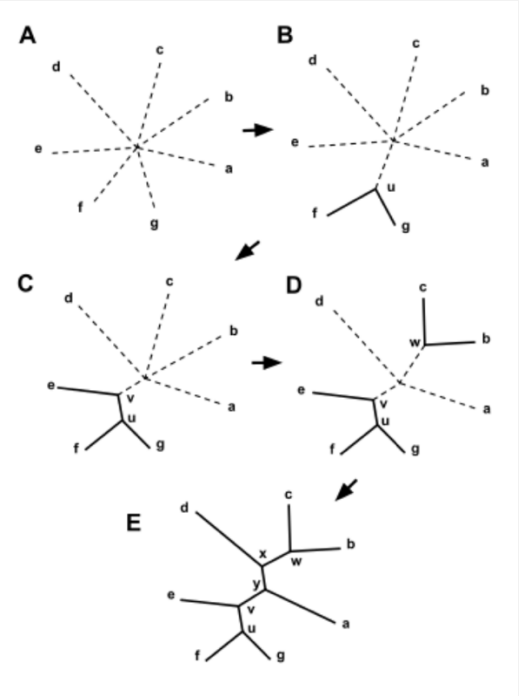
Simple methods (Neighbour-Joining)

- Neighbor-Joining (NJ) is a distance-based method used to construct phylogenetic trees
- Starts with a star-like tree, where all entities are connected to a central node (A)
- The Neighbor-Joining algorithm iteratively joins entities (nodes) in the tree while minimizing the total branch length



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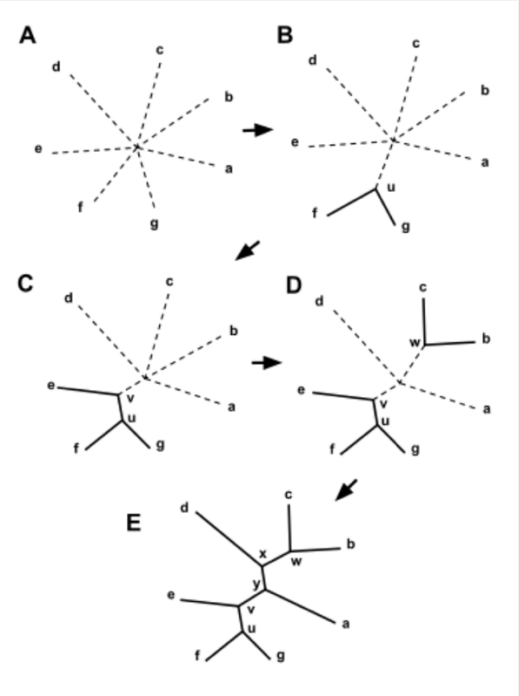
Simple methods (Neighbour-Joining)

- Neighbor-Joining is a relatively efficient and versatile method for constructing phylogenetic trees
- However, it does not explicitly model the underlying evolutionary processes, such as substitutions, insertions, deletions, or other events
- This limitation makes it less suitable for analyzing complex evolutionary scenarios



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Evolutionary models

- More sophisticated tree building methods can include models:
  - Nucleotide substitution models
  - Molecular clock models
  - Population models
  - Coalescent models

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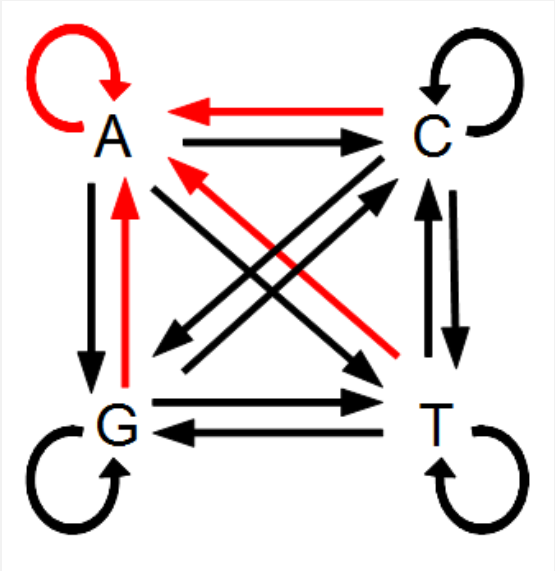
Nucleotide substitution models

- Describe the rates at which nucleotides in DNA sequences change over time
- Provide a framework for estimating the likelihood of observed DNA sequence data on a phylogenetic tree
- Common models include:
  - Jukes-Cantor (JC) Model
  - Hasegawa-Kishino-Yano (HKY) Model
  - General Time Reversible (GTR) Model



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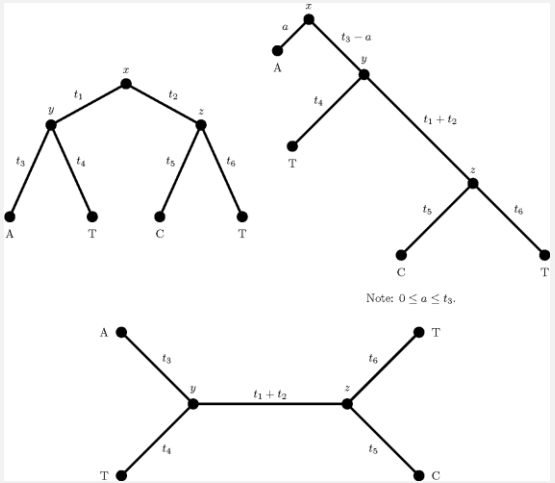
More complex methods (Maximum Likelihood)

- ML tree construction aims to find the tree topology and branch lengths that maximize the likelihood of the observed sequence data under a specified evolutionary model
- ML is powerful for its statistical rigor and ability to handle complex models of evolution
- Generally considered less flexible in handling complex models compared to Bayesian methods



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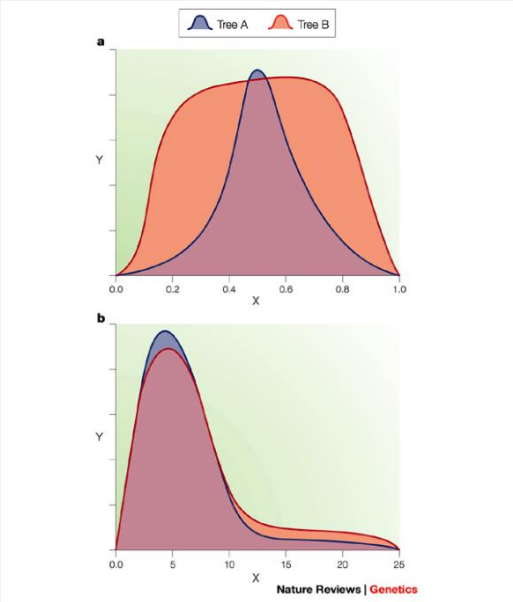
Bayesian phylogenies

- Uses Bayesian statistics to estimate the posterior probabilities of different phylogenetic trees, incorporating the observed data and prior knowledge
- Calculates the probabilities of different trees by combining the likelihood of the observed data with the prior probability distributions over tree space, based on certain models of evolution
- Require careful selection of priors and are computationally demanding.



From Holder & Lewis, 2003

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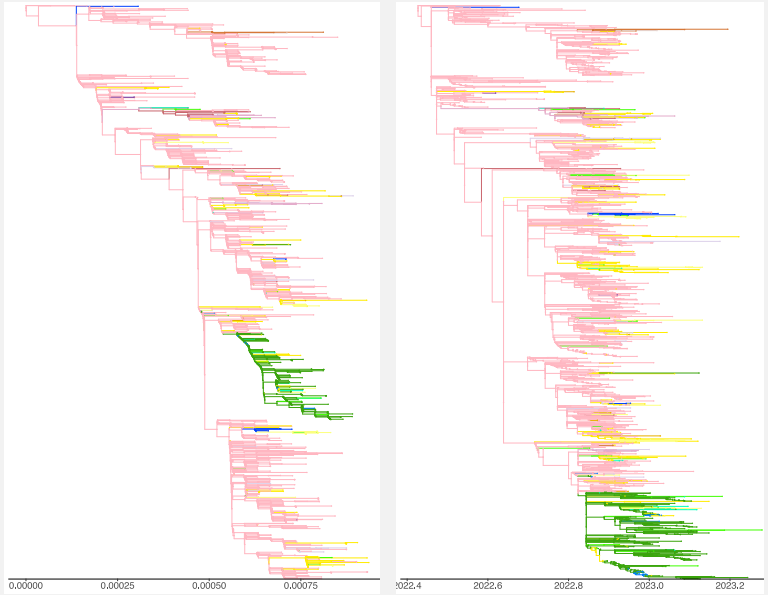
Timed vs untimed trees

- In untimed trees, branch lengths typically represent genetic or sequence divergence
- Help to understand the evolutionary ancestry and genetic relatedness among taxa
- Timed trees incorporate the estimated timing of evolutionary events, branches are scaled to unit time
- Useful for estimating divergence times, studying temporal changes in evolutionary processes, and reconstructing the evolutionary history of lineages



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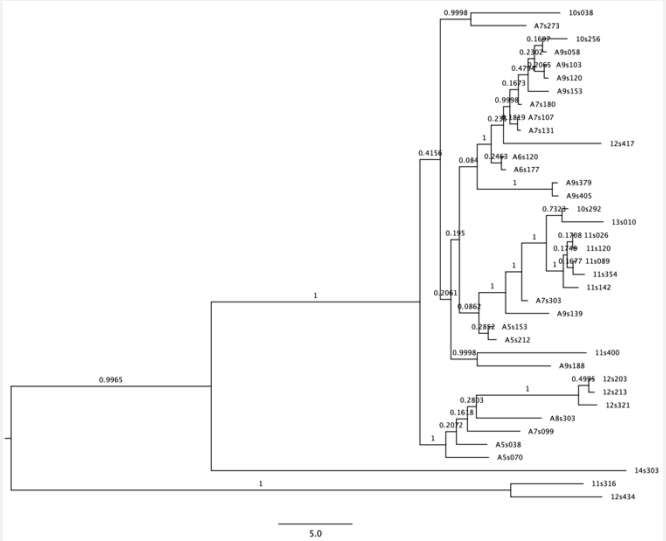
Assessing phylogenetic trees

- Bootstrapping is a resampling technique used in phylogenetics to assess the robustness of the inferred phylogenetic tree topology
- It can estimate the reliability of the branching patterns in a phylogenetic tree
- Resamples data and builds multiple trees to assign support values to branches and nodes
- High bootstrap support values indicate the inferred relationships are likely to be accurate



PRACTICAL 2: VARIANT CALLING AND MAXIMUM LIKELIHOOD TREES

1. Variant calling and VCFs
2. Building consensus sequences
3. Aligning consensus sequences
4. Maximum Likelihood trees