A (short) introduction to phylogenetics

Thibaut Jombart

MRC Centre for Outbreak Analysis and Modelling
Imperial College London

Genetic data analysis with \textit{R}

PR\textasciitilde Statistics, Glasgow
03-08-2015
Outline

Context

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

And more...
Outline

Context

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

And more...
'From the first growth of the tree, many a limb and branch has decayed and dropped off; and these fallen branches of various sizes may represent those whole orders, families, and genera which have now no living representatives, and which are known to us only in a fossil state.'

C. Darwin, Notebook, 1837.
Phylogenetics: ...to the present

- phylogenetic trees are part of the standard toolbox of genetic data analysis
- represent the evolutionary history of a set of (sampled) taxa

Bininda-Emonds et al., 2007, Nature.
And the main difference is...

Current trees look better!

(and some other minor differences)
And the main difference is...

Current trees look better!

(and some other minor differences)
And the main difference is...

Current trees look better!

(and some other minor differences)
About the minor differences...

- DNA sequencing revolution
- huge data banks freely available (e.g. GenBank)
- easier, cheaper, faster to obtain DNA sequences
- increasing number of full genomes available

Different ways to exploit this information.
About the minor differences...

- DNA sequencing revolution
- huge data banks freely available (e.g. GenBank)
- easier, cheaper, faster to obtain DNA sequences
- increasing number of full genomes available

Different ways to exploit this information.
Outline

Context

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

And more...
Phylogenetic trees: some useful terms

**Phylogenetic tree**: representation of evolutionary relationships between a set of taxa.

- Nodes
- Hypothetical Taxonomic Units (HTUs)
- Most Recent Common Ancestors (MRCA)

- Tips
- Leaves
- Operational Taxonomic Units (OTUs)

- branch
- edge

root
Accumulated substitutions tell us about the genealogy

**Substitution**: replacement of a nucleotide (e.g. $a \rightarrow t$)
Accumulated substitutions tell us about the genealogy

**Substitution**: replacement of a nucleotide (e.g. $a \rightarrow t$)
Accumulated substitutions tell us about the genealogy

**Substitution**: replacement of a nucleotide (e.g. $a \rightarrow t$)
Accumulated substitutions tell us about the genealogy

**Substitution**: replacement of a nucleotide (e.g. $a \rightarrow t$)
From alignments to phylogenies

...attaaacgtaggatctagg...
...attaaacgtaggatctagg...
...attcatacgtaggatcagg...
...attgtacgtaggatctttt...
...attgtacgtaggatctttt...
...attgcatgtaggatctttt...
From alignments to phylogenies

...attaaacgtaggatctagg...
...attaaacgtaggatctagg...
...attcatacgtaggatcagg...
...attgtacgtaggatctttt...
...attgtacgtaggatctttt...
...attgcatgtaggatctttt...
...attgcatgtaggatctttt...

Reconstructed phylogeny
From alignments to phylogenies

Different methods for achieving phylogenetic reconstruction.
Workflow

Prepare data

- align sequences: alignment software + manual refinement

Build the tree

- distance-based methods
- maximum parsimony
- likelihood-based methods (ML, Bayesian)

Analyse the tree

- assess uncertainty
- test phylogenetic signal
- model trait evolution
- …
Workflow

Prepare data

• align sequences: alignment software + manual refinement

Build the tree

• distance-based methods
• maximum parsimony
• likelihood-based methods (ML, Bayesian)

Analyse the tree

• assess uncertainty
• test phylogenetic signal
• model trait evolution
• …
Workflow

Prepare data

• align sequences: alignment software + manual refinement

Build the tree

• distance-based methods
• maximum parsimony
• likelihood-based methods (ML, Bayesian)

Analyse the tree

• assess uncertainty
• test phylogenetic signal
• model trait evolution
• ...
Workflow

Prepare data

- align sequences: alignment software + manual refinement

Build the tree

- distance-based methods
- maximum parsimony
- likelihood-based methods (ML, Bayesian)

Analyse the tree

- assess uncertainty
- test phylogenetic signal
- model trait evolution
- ...
Outline

Context

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

And more...
Distance-based phylogenetic reconstruction

Approaches relying on **agglomerative clustering** algorithms (e.g. Single linkage, UPGMA, Neighbor-Joining)

**Rationale**

1. compute pairwise genetic distances $D$
2. group closest sequences
3. update $D$
4. go back to 2) until all sequences are grouped
Distance-based phylogenetic reconstruction

Alignment

...attaaacgtaggatctagg...
...attaaacgtaggatctagg...
...attcatacgtaggatctagg...
...attgtacgtaggatcttttt...
...attgtacgtaggatcttttt...
...attgtacgtaggatcttttt...
...attgcatgtaggatcttttt...
Distance-based phylogenetic reconstruction

Alignment

...attaacgtaggatctagg...
...attaacgtaggatctagg...
...ttcatacgtaggatcagg...
...attgtacgtaggatctttt...
...attgtacgtaggatctttt...
...attgtacgtaggatctttt...
...attgcgtgtaggatctttt...

Distance matrix
Distance-based phylogenetic reconstruction

Alignment

...attaacgtaggatctagg...
...attaacgtaggatctagg...
...ttccatacgtaggatcagg...
...attgtacgtaggatctttt...
...attgtacgtaggatttttt...
...attgtacgtaggatctttt...
...attgcatgttaggatcttttt...

Distance matrix
Distance-based phylogenetic reconstruction

Alignment

...attaacgtagaatctagg...
...attaacgtagaatctagg...
...attcatacgtagatcagg...
...attgtacgtagatctttt...
...attgtacgtagatctttt...
...attgtacgtagatctttt...
...attgtacgtagatctttt...
...attgtacgtagatctttt...

Distance matrix
Distance-based phylogenetic reconstruction

Alignment

...attaacgtagtatcctagg...
...attaacgtagtatcctagg...
...attcaacgttagatcctagg...
...attgtacgtagatcttttt...
...attgtacgtagatcttttt...
...attgtacgtagatcttttt...
...attgtacgtagatcttttt...

Distance matrix
Distance-based phylogenetic reconstruction

Alignment

...atataacgtagatctagg...
...atataacgtagatctagg...
...attcatacgtaggatcagg...
...attgtacgttagatctttt...
...attgtacgttagatctttt...
...attgtacgttagatctttt...
...attgtacgttagatctttt...
...attgtacgttagatctttt...

Distance matrix

Reconstructed tree
What is the distance between a node and tips?

Hierarchical clustering:

\[ D_{k,g} = \ldots \]

- single linkage: \( D_{k,g} = \min(D_{k,i}, D_{k,j}) \)
- complete linkage: \( D_{k,g} = \max(D_{k,i}, D_{k,j}) \)
- UPGMA: \( D_{k,g} = \frac{D_{k,i} + D_{k,j}}{2} \)

Neighbor joining:
Transforms original distances to account for heterogeneous rates of evolution.
What is the distance between a node and tips?

Hierarchical clustering:

- single linkage: \( D_{k,g} = \min(D_{k,i}, D_{k,j}) \)
- complete linkage: \( D_{k,g} = \max(D_{k,i}, D_{k,j}) \)
- UPGMA: \( D_{k,g} = \frac{D_{k,i} + D_{k,j}}{2} \)

Neighbor joining:
Transforms original distances to account for heterogeneous rates of evolution.
What is the distance between a node and tips?

Hierarchical clustering:

- single linkage: $D_{k,g} = \min(D_{k,i}, D_{k,j})$
- complete linkage: $D_{k,g} = \max(D_{k,i}, D_{k,j})$
- UPGMA: $D_{k,g} = \frac{D_{k,i} + D_{k,j}}{2}$

Neighbor joining:
Transforms original distances to account for heterogeneous rates of evolution.
What is the distance between a node and tips?

Hierarchical clustering:

- single linkage: \( D_{k,g} = \min(D_{k,i}, D_{k,j}) \)
- complete linkage: \( D_{k,g} = \max(D_{k,i}, D_{k,j}) \)
- UPGMA: \( D_{k,g} = \frac{D_{k,i} + D_{k,j}}{2} \)

Neighbor joining: Transforms original distances to account for heterogeneous rates of evolution.
What is the distance between a node and tips?

Hierarchical clustering:

- single linkage: $D_{k,g} = \min(D_{k,i}, D_{k,j})$
- complete linkage: $D_{k,g} = \max(D_{k,i}, D_{k,j})$
- UPGMA: $D_{k,g} = \frac{D_{k,i} + D_{k,j}}{2}$

Neighbor joining:
Transforms original distances to account for heterogeneous rates of evolution.
Distance-based phylogenetic reconstruction

Advantages

• simple
• flexible (many distances and clustering algorithms)
• fast and scalable (applicable to large datasets)

Limitations

• sensitive to distance/clustering chosen
• evolutionary rates are not estimated
• no measure of uncertainty for the tree obtained
Distance-based phylogenetic reconstruction

Advantages

- simple
- flexible (many distances and clustering algorithms)
- fast and scalable (applicable to large datasets)

Limitations

- sensitive to distance/clustering chosen
- evolutionary rates are not estimated
- no measure of uncertainty for the tree obtained
Outline

Context

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

And more...
Maximum parsimony phylogenies

Approaches relying on finding the tree with the smallest number of character changes (substitutions)

Rationale

1. start from a pre-defined tree
2. compute initial parsimony score
3. permute branches and compute parsimony score
4. accept new tree if the parsimony score is improved
5. go back to 3) until convergence
Maximum parsimony phylogenies

Initial tree

score: 6
Maximum parsimony phylogenies
Maximum parsimony phylogenies

Initial tree

score: 6

score: 6

score: 8
Maximum parsimony phylogenies

Initial tree

Score: 6

Score: 8

Score: 5
Maximum parsimony phylogenies

Initial tree

score: 6

score: 5

score: 8
Maximum parsimony phylogenies

Advantages

• applicable to any discontinuous characters (not just DNA)
• intuitive explanation: ‘simplest’ evolutionary scenario

Limitations

• evolutionary rates are not estimated
• no measure of uncertainty for the tree obtained
• computer-intensive
• different types of substitutions ignored
• evolution not necessarily parsimonious
• sensitive to heterogeneous rates of evolution (long branch attraction)
Maximum parsimony phylogenies

Advantages

- applicable to any discontinuous characters (not just DNA)
- intuitive explanation: ‘simplest’ evolutionary scenario

Limitations

- evolutionary rates are not estimated
- no measure of uncertainty for the tree obtained
- computer-intensive
- different types of substitutions ignored
- evolution not necessarily parsimonious
- sensitive to heterogeneous rates of evolution (long branch attraction)
Outline

Context

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

And more...
Likelihood-based phylogenies (ML / Bayesian)

Approaches relying on a **model of sequence evolution**:

- **ML**: find tree and evolutionary rates with highest likelihood
- **Bayesian**: find tree and evolutionary rates to posterior probability

**Rationale**

1. start from a pre-defined tree
2. compute initial likelihood/posterior
3. permute branches, sample new parameters and compute likelihood/posterior
4. accept new tree and parameters based on likelihood/posterior improvement
5. go back to 3) until convergence
Likelihood-based phylogenies (ML / Bayesian)

Approaches relying on a **model of sequence evolution**:

- **ML**: find tree and evolutionary rates with highest likelihood
- **Bayesian**: find tree and evolutionary rates to posterior probability

**Rationale**

1. start from a pre-defined tree
2. compute initial likelihood/posterior
3. permute branches, sample new parameters and compute likelihood/posterior
4. accept new tree and parameters based on likelihood/posterior improvement
5. go back to 3) until convergence
Likelihood-based phylogenies (ML / Bayesian)

Density

Likelihood / Posterior

Uncertainty

Parsimony

Pitfalls & best practices

And more...
Likelihood-based phylogenies (ML / Bayesian)

Advantages

• very flexible
• consistent with a model of evolution
• statistically consistent (model comparison)
• parameter estimation
• (Bayesian) several trees → measure of uncertainty

Limitations

• computer-intensive
• choice of the model of evolution
• (ML) no measure of uncertainty for the tree obtained
• (Bayesian) need to find a consensus tree
Likelihood-based phylogenies (ML / Bayesian)

Advantages

- very flexible
- consistent with a model of evolution
- statistically consistent (model comparison)
- parameter estimation
- (Bayesian) several trees $\rightarrow$ measure of uncertainty

Limitations

- computer-intensive
- choice of the model of evolution
- (ML) no measure of uncertainty for the tree obtained
- (Bayesian) need to find a consensus tree
Outline

Context

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

And more...
How do we know the tree is robust?

**Main issue:** assess the uncertainty of the tree topology / individual nodes

**Approaches**

- ML: model selection to compare trees (whole tree)
- Bayesian methods: between-samples variability (individual nodes)
- any method: bootstrap (individual nodes)
How do we know the tree is robust?

**Main issue:** assess the uncertainty of the tree topology / individual nodes

**Approaches**

- **ML:** model selection to compare trees (whole tree)
- **Bayesian methods:** between-samples variability (individual nodes)
- **any method:** bootstrap (individual nodes)
How do we know the tree is robust?

**Main issue:** assess the uncertainty of the tree topology / individual nodes

**Approaches**

- ML: model selection to compare trees (whole tree)
- Bayesian methods: between-samples variability (individual nodes)
- any method: bootstrap (individual nodes)
How do we know the tree is robust?

**Main issue:** assess the uncertainty of the tree topology / individual nodes

**Approaches**

- ML: model selection to compare trees (whole tree)
- Bayesian methods: between-samples variability (individual nodes)
- any method: bootstrap (individual nodes)
How do we know the tree is robust?

**Main issue:** assess the uncertainty of the tree topology / individual nodes

**Approaches**

- **ML:** model selection to compare trees (whole tree)
- **Bayesian methods:** between-samples variability (individual nodes)
- **any method:** bootstrap (individual nodes)
Bootstrapping phylogenies

- assess **variability due to sampling the genome and conflicting signals**
- relies on analysing **resampled datasets**

**Rationale**

1. obtain a reference tree
2. resample the sites with replacement
3. obtain a tree for the resampled dataset
4. go back to 2) until the desired number of bootstrapped trees is attained
5. compute the frequency of each bifurcation of the reference tree occurring in bootstrapped trees
Bootstrapping phylogenies

- assess **variability due to sampling the genome and conflicting signals**
- relies on analysing **resampled datasets**

**Rationale**

1. obtain a reference tree
2. resample the sites with replacement
3. obtain a tree for the resampled dataset
4. go back to 2) until the desired number of bootstrapped trees is attained
5. compute the frequency of each bifurcation of the reference tree occurring in bootstrapped trees
Bootstrapping phylogenies

Reconstructed phylogeny

Sampling sites with replacement

Compare topologies

Reconstructed phylogeny

...attaaacgtaggatcttagg...
...attaaacgtaggatcttagg...
...attctacgtaggatcagg...
...attgtacgtaggatctttt...
...attgtacgtaggatctttt...
...attgcattaggatctttt...

...ttttaaccatgatcttagg...
...ttttaaccatgatcttagg...
...attctacgtaggatcagg...
...aaatgtaccatgatctttt...
...aaatgtaccatgatctttt...
...aaatgcatcatgatctttt...

...ttttaaccatgatcttagg...
...ttttaaccatgatcttagg...
...attctacgtaggatcagg...
...aaatgtaccatgatctttt...
...aaatgtaccatgatctttt...
...aaatgcatcatgatctttt...

29/39
Bootstrapping phylogenies

Advantages

• standard
• simple to implement

Limitations

• possibly computer-intensive
• assumes that the genome has been sampled randomly (often wrong)
Bootstrapping phylogenies

Advantages

- standard
- simple to implement

Limitations

- possibly computer-intensive
- assumes that the genome has been sampled randomly (often wrong)
Outline

Context

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

And more...
Never plot an unrooted tree as rooted.
Plotting trees as rooted

Never plot an unrooted tree as rooted.
Interpreting distances
Interpreting distances
Interpreting distances

meaningful distance = sum of branch lengths
The paradox of divergent clusters

MRCA and genetic distances may give different information.
The paradox of divergent clusters

\[ D(\text{red}, \text{red}) > D(\text{red}, \text{blue}) \]

MRCA and genetic distances may give different information.
The paradox of divergent clusters

\[ D(\text{red, red}) > D(\text{red, blue}) \]

MRCA and genetic distances may give different information.
At best, the tree is an estimate of the likely evolutionary history of the taxa studied.
Taking uncertainty into account

193 HIV−1 sequences from DRC (Strimmer & Pybus 2001)

At best, the tree is an estimate of the likely evolutionary history of the taxa studied.
Taking uncertainty into account

At best, the tree is an estimate of the likely evolutionary history of the taxa studied.
(Over, Mis)Interpreting temporal trends

“Time trees” only make sense under a near-perfect molecular clock.
(Over, Mis)Interpreting temporal trends

"Time trees" only make sense under a near-perfect molecular clock.
"Time trees" only make sense under a near-perfect molecular clock.
Outline

Context

Phylogenies...

Distance trees

Parsimony

Likelihood/Bayesian

Uncertainty

Pitfalls & best practices

And more...
This is only the beginning

Many things can be done with trees

- estimate divergence time
- model trait evolution (phylogenetic comparative method)
- reconstruct ancestral states
- measure diversity
- infer past demographics/effective population size (coalescence)
- ...

- and also, other approaches than phylogenetics to analyse genetic data
This is only the beginning

Many things can be done with trees

- estimate divergence time
- model trait evolution (phylogenetic comparative method)
- reconstruct ancestral states
- measure diversity
- infer past demographics/effective population size (coalescence)
- ... 
- and also, other approaches than phylogenetics to analyse genetic data
This is only the beginning

Many things can be done with trees

- estimate divergence time
- model trait evolution (phylogenetic comparative method)
- reconstruct ancestral states
- measure diversity
- infer past demographics/effective population size (coalescence)
- ...
- and also, other approaches than phylogenetics to analyse genetic data
This is only the beginning

Many things can be done with trees

- estimate divergence time
- model trait evolution (phylogenetic comparative method)
- reconstruct ancestral states
- measure diversity
- infer past demographics/effective population size (coalescence)
- ...
- and also, other approaches than phylogenetics to analyse genetic data
This is only the beginning

Many things can be done with trees

- estimate divergence time
- model trait evolution (phylogenetic comparative method)
- reconstruct ancestral states
- measure diversity
- infer past demographics/effective population size (coalescence)
- ...

- and also, other approaches than phylogenetics to analyse genetic data
This is only the beginning

Many things can be done with trees

- estimate divergence time
- model trait evolution (phylogenetic comparative method)
- reconstruct ancestral states
- measure diversity
- infer past demographics/effective population size (coalescence)
- ...
- and also, other approaches than phylogenetics to analyse genetic data
This is only the beginning

Many things can be done with trees

- estimate divergence time
- model trait evolution (phylogenetic comparative method)
- reconstruct ancestral states
- measure diversity
- infer past demographics/effective population size (coalescence)
- ...

- and also, other approaches than phylogenetics to analyse genetic data
Time to get your hands dirty!

The pdf of the practical is online:

http://adegenet.r-forge.r-project.org/

or

Google → adegenet → documents → “Workshop Glasgow, August 2015”