Phylogenomic inference with IQ-TREE

Stephen Crotty

Cesky Krumlov, Czech Republic, 24 Jan 2019
Typical phylogenetic analysis

Sequence alignment

<table>
<thead>
<tr>
<th>Sequence 1</th>
<th>Sequence 2</th>
<th>Sequence 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACCTGTCGT</td>
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<td>CAGCTGTCGT</td>
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</tbody>
</table>

Substitution models

Model selection

Tree search

Bootstrap

Phylogeny
Models of sequence evolution

Rate heterogeneity: sites evolve at different rates - some slow, some fast.

<table>
<thead>
<tr>
<th>Rate model</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+I</td>
<td>Some sites are invariable (zero rate), e.g. due to selective force.</td>
</tr>
<tr>
<td>+G</td>
<td>Site rates follow a Gamma distribution.</td>
</tr>
<tr>
<td>+I+G</td>
<td>Some sites are invariable, the rest follow a Gamma distribution.</td>
</tr>
<tr>
<td>+R</td>
<td>Sites fall into several categories from slow to fast rates. No assumption of rate distribution (free-rate model).</td>
</tr>
</tbody>
</table>

A model = substitution model + rate heterogeneity, e.g. “GTR+G”
Model selection

Which model is best?

**Problem:**
More complex models always have higher *likelihood* than simpler models!

**Solution:** Penalize a model $M$ by the number of its parameters ($k$)

1. Akaike information criterion (AIC): 
   \[ AIC = 2k - 2\ln(L(D|T, M)) \]

2. Bayesian information criterion (BIC): 
   \[ BIC = \ln(n)k - 2\ln(L(D|T, M)), \]
   where $n$ is the number of alignment sites.

Select the model with smallest AIC or BIC score.
UFBoot: Ultrafast bootstrap approximation

Alignment

**ML tree search with the IQ–TREE strategy**

- Many trees collected during tree search with their estimated site log–likelihoods

- Tree 1
  - AGGCTCCT
  - AGGTTCCGC
  - AGGCCCGGT
  - ATTTCCGA

- Tree 2
  - AGGCTCCT
  - AGGTTCCGC
  - AGGCCCGGT
  - ATTTCCGA

- Tree x
  - AGGCTCCT
  - AGGTTCCGC
  - AGGCCCGGT
  - ATTTCCGA

Estimated site log–likelihoods from the original alignment

**Relaxing Estimated site Log–Likelihoods (RELL)**

- RELL sample 1 for tree 1
- RELL sample 1 for tree 2
- RELL sample 1 for tree x

- RELL sample 2 for tree 1
- RELL sample 2 for tree 2
- RELL sample 2 for tree x

**Map branch proportions onto ML tree**

- Best RELL–trees

Use UFBoot >= 95% instead of 70%!
Practical: Where is Turtle in the tree?

Dataset: 16 species, 29 genes, 20,820 bp (a subset of Chiari et al. 2012)

Different studies led to different trees!

Thanks Jeremy Brown
1. Input data
2. Inferring the first phylogeny
3. Applying partition model
4. Choosing the best partitioning scheme
5. Applying a mixture model - GHOST
6. Tree topology tests
7. Concordance factors
8. Identifying most influential genes

http://www.iqtree.org/workshop/ck2019
Genome-scale data

### Phylogenomic Inference

Species tree of life

<table>
<thead>
<tr>
<th>Gene 1</th>
<th>Gene 2</th>
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<th>Gene 1,000</th>
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<tr>
<td>CACCTGTCGT</td>
<td>--------</td>
<td></td>
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<td>CTGAGCCTTG</td>
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</table>

30 days of computation and 280 GB RAM for an insect data set (CSIRO)!
Exercises

1. Input data
2. Inferring the first phylogeny
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http://www.iqtree.org/workshop/ck2019
“Data-model gap” is increasing!

Level of model violations in 35 phylogenomic datasets (https://doi.org/10.1101/460121)

1. Resulting trees tend to be biased towards the genes that violated model assumptions.
2. Bootstrap supports tend to 100% as #genes increases.

Model violation → Systematic bias

1. Remove “bad” loci
2. Use more realistic models
Partition model

Substitution models:

- JC
- HKY+G
- ...... (omitted)
- GTR+G

Recommended for typical analysis, confirmed by Dunchene et al. (2018)

https://doi.org/10.1101/467449
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How to reduce potential model overfitting?

**Supermatrix**

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**PartitionFinder**

Gene 1+2          | ...          | Gene 200+1,000

| CACCTGTCGT      | ------------ | ...          | TCTGGTGCAG |
| CAGCTGTCGT      | GCTCTTTCTG  | TTGAGCCCTGG  | TCTGGTGCAAG|
| CAGCTGCCGT      | GTTCTTCTCG  | TTGAGCCCTGG  | TCTGGTGCAAG|
| CAGCTGCCGG      | GTCCTCTCGG  | ------------  | TCTGGTGCAA |
| CTCCTGCCGG      | GTCCTCTCAG  | ------------  |            |
| CTCCTGCCGG      | ------------ | CTGAGCCGGG   | TCTGGTGCAAG|
| CTCTTGGCCGG     | ------------ | CTGAGCCCTTG  |            |

**PartitionFinder algorithm** (Lanfear et al. 2012):

1. Evaluate to merge all pairs of genes.
2. Choose the pair with the best score.
3. If score improves, merge two genes and repeat steps 1-3.
4. Otherwise, stop.

**Relaxed clustering algorithm** (Lanfear et al. 2014):

In step 1: only examine the top k% of most “promising” pairs.

Substitution models: HKY ...... GTR+G
Exercises

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4. **Choosing the best partitioning scheme**
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http://www.iqtree.org/workshop/ck2019
Mixture model - GHOST

Partition Model

Gene 1                  Gene 2                  ......                  Gene 1,000
CACCTGTCGT              -----------              -----------              TCTGGTGCAG
CAGCTGTCGT              GCTCTTTTCTG          TTAGGCTCTGG          TCTGGTGCAG
CAGCTGCCGG              GTTTTTCTCTG          TTAGGCTCTGG          TCTGGTACAG
CAGCTGCCGG              GTTCTCTCCTG          TCTGGTGCAG
CTCCTGCCGG              -----------              -----------              -----------
CTCCTGCCGG              -----------              CTGAGCCGGG          TCTGGTGCAG
CTCTTGGCCGG             -----------              CTGAGCCCTTGG         -----------

Mixture Model

Gene 1                  Gene 2                  ......                  Gene 1,000
CACCTGTCGT              -----------              -----------              TCTGGTGCAG
CAGCTGTCGT              GCTCTTTTCTG          TTAGGCTCTGG          TCTGGTGCAG
CAGCTGCCGG              GTTTTTCTCTG          TTAGGCTCTGG          TCTGGTACAG
CAGCTGCCGG              GTTCTCTCCTG          TCTGGTGCAG
CTCCTGCCGG              -----------              -----------              -----------
CTCCTGCCGG              -----------              CTGAGCCGGG          TCTGGTGCAG
CTCTTGGCCGG             -----------              CTGAGCCCTTGG         -----------

\[ \mathcal{L}(s_{ik} | \mathcal{M}) = \sum_{j=1}^{m} \delta_{kj} \mathcal{L}_{ij}(s_{ik} | M_j, T, \lambda_j) \]

\[ \mathcal{L}(s_i | \mathcal{M}) = \sum_{j=1}^{m} w_j \mathcal{L}_{ij}(s_i | M_j, T, \lambda_j) \]

For full details of the GHOST model see Crotty et al. (2019)
https://doi.org/10.1101/174789
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http://www.iqtree.org/workshop/ck2019
Testing two trees (Kishino & Hasegawa, 1989):

1. Statistic: \( \Delta = \ln(L(D \mid T_1)) - \ln(L(D \mid T_2)) \)
2. Generate distribution of \( \Delta \) from many “random” data (e.g. by 1000 bootstrap resampling).
3. Compare the statistic between original and random data to obtain \( p\)-value.
4. If \( p\)-value < 0.05: YES! two trees are significantly different.
5. If \( p\)-value \( \geq \) 0.05: NO! they are not.
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http://www.iqtree.org/workshop/ck2019
Inferring species tree from gene trees

**Supermatrix**

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Gene tree 1  Gene tree 2  ..........  Gene tree 1,000

Species tree
Concordance factor

**Gene concordance factor (gCF):**
How often each branch of species tree is found among the gene trees?

**Site concordance factor (sCF):**
How often each branch of species tree is supported by the alignment sites?

- gCF and sCF captures dis/agreement in the data, whereas bootstrap doesn’t!
- gCF and sCF are useful when bootstrap supports reach 100%.

Sequence alignment:

<table>
<thead>
<tr>
<th></th>
<th>Genes 1</th>
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<th>Genes 1000</th>
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<tr>
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Gene boundaries are obvious within the dataset, as most genes are not present for all species.

The four turtle (emys, phrynops, caretta, chelonodis) and two crocodile (caiman, alligator) species have much more missing data than most other species in the alignment. This might make their position in the tree more difficult to resolve.
The best-fit model found by ModelFinder was GTR+F+R3. This means:
• the GTR model of sequence evolution
• base frequencies calculated empirically from the alignment (as opposed to inferred under ML)
• three categories of rate heterogeneity, with rates and weights inferred by ML, not constrained to the Gamma distribution.
Tree inferred under GTR+F+R3 without partitioning the alignment. Turtles (red) are sister to crocodiles (blue), in contradiction with the published tree, in which turtles are sister to archosaurs (crocodiles and birds).
• The slowest evolving gene is the 10th gene, with a rate of 0.4683.

• The fastest evolving gene is the 18th gene, with a rate of 1.8421.

• The BIC of the non-partitioned model is 232837.7889.

• The BIC of the partitioned model is 233126.4205.

Even though the partitioned model has a higher likelihood than the non-partitioned model, the non-partitioned model has a smaller (better) BIC, and on that basis should be preferred. This is because the partitioned model has 221 free parameters, compared to just 41 for the non-partitioned model.
Tree inferred under the partition model. Turtles (red) are sister to archosaurs (blue), concurring with the published tree.
• The BIC of the non-partitioned model is 232837.7889.

• The BIC of the partitioned model is 233126.4205.

• The BIC of the best partition model is 232401.3940

By merging similar genes, we have reduced the number of partitions from 29 to 10. This has reduced the number of parameters in the model from 221 to 106 and consequently, the best partition scheme now has the lowest BIC score of the three models considered so far.
Tree inferred under best partition scheme. This topology agrees with that inferred by the non-partitioned model, and conflicts with the published tree. The bootstrap support for the conflicting branch has fallen from 83 to 70.
Tree inferred under GHOST model. This topology agrees with the inferred tree under the full partition model, and the published tree. Compared to the full partition model, the bootstrap support for the contentious branch has increased from 71 to 93.
**USER TREES**

See `turtle.test.trees` for trees with branch lengths.

<table>
<thead>
<tr>
<th>Tree</th>
<th>logL</th>
<th>deltaL</th>
<th>bp-RELL</th>
<th>p-KH</th>
<th>p-SH</th>
<th>c-ELW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-115476.8396</td>
<td>6.7446</td>
<td>0.399 +</td>
<td>0.394 +</td>
<td>0.394 +</td>
<td>0.401 +</td>
</tr>
<tr>
<td>2</td>
<td>-115470.095</td>
<td>0</td>
<td>0.601 +</td>
<td>0.606 +</td>
<td>1 +</td>
<td>0.599 +</td>
</tr>
</tbody>
</table>

`deltaL` : logL difference from the maximal logl in the set.
`c-ELW` : Expected Likelihood Weight (Strimmer & Rambaut 2002).

Plus signs denote the 95% confidence sets.
Minus signs denote significant exclusion.
All tests performed 1000 resamplings using the RELL method.
Inferred tree for the full partition model. The node annotation indicates BS/GCF/SCF scores. The contentious branch splitting turtles and archosaurs has GCF of 37.9%, which equates to 11 of the 29 genes supporting this topology.
Inferred tree for the non-partition model. The node annotation indicates BS/GCF/SCF scores. The contentious branch splitting turtles and archosaurs has GCF of 17.2%, which equates to 5 of the 29 genes supporting this topology.
When we examine the difference in log-likelihoods between the two trees on a per-gene basis, we notice that two particular genes strongly support turtles as sister to crocodiles, whereas most other genes are either neutral, or support turtles as sister to archosaurs.