Phylogenomics

Gene trees/species tree

Tomáš Fér

Biosystematics, 2018

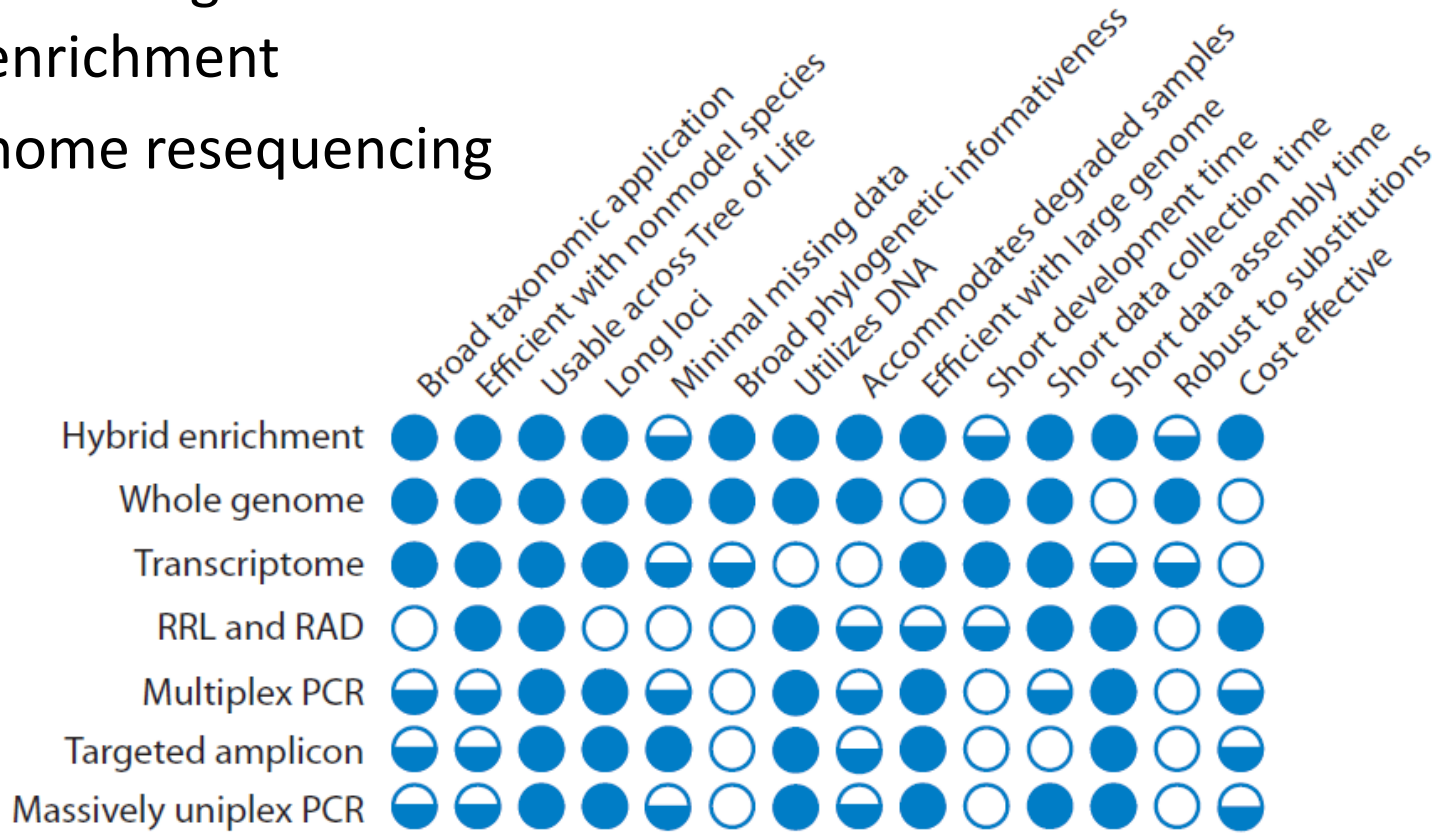
Phylogenomics

- using whole-genome sequences or large portion of the genome to build a phylogeny
  - whole chloroplast sequences
  - hundreds or thousands of genes
    - transcriptomes
    - target-enrichment

- gene tree – individual evolutionary history
- species tree – ‘true’ species evolution
- gene tree/species tree

Phylogenomic data sources

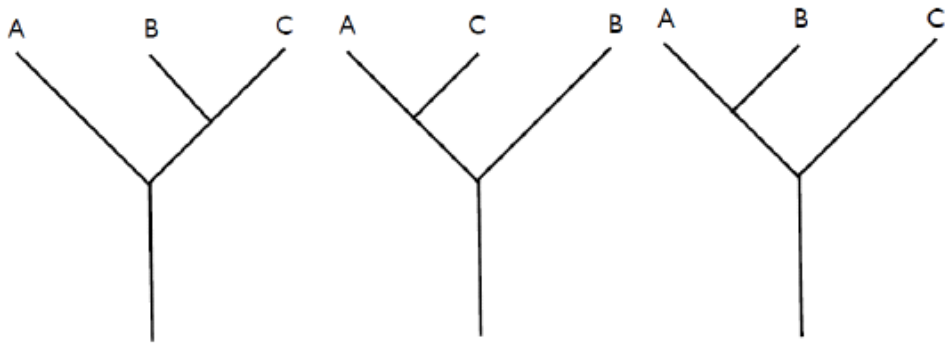
- transcriptomes
- genome skimming
- targeted enrichment
- whole genome resequencing



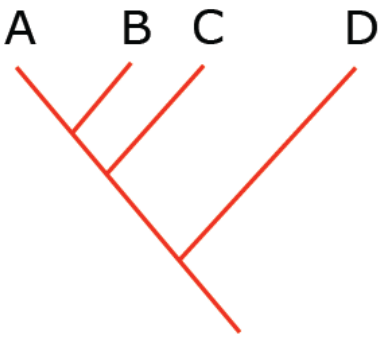
Lemmon E.M. & Lemmon A.R. (2013): *High-throughput genomic data in systematics and phylogenetics*. Annu. Rev. Ecol. Evol. Syst, 44, 99–121.

Incongruencies among loci: gene trees vs species tree

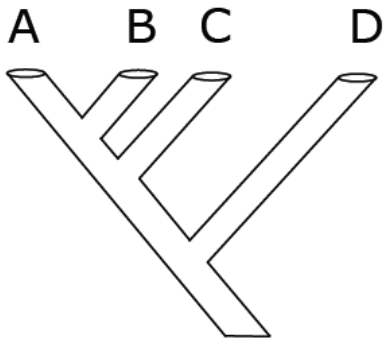
- gene duplications and losses (orthology problem)
- incomplete lineage sorting/deep coalescence
- hybridization
- polyploidization
- recombination



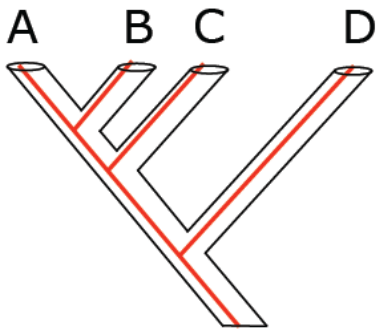
Gene trees vs species tree



Gene tree

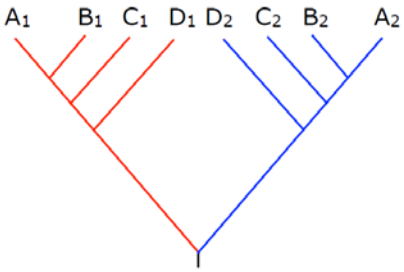


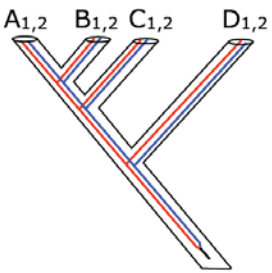
Species tree

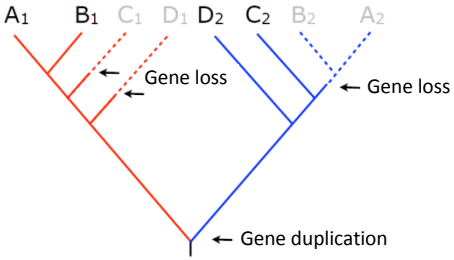


Gene tree = Species tree

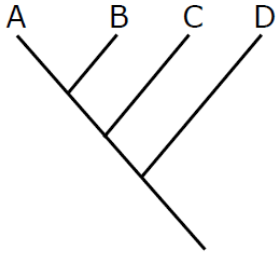
gene duplications and losses

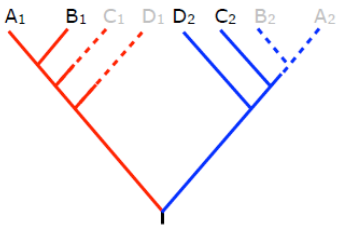




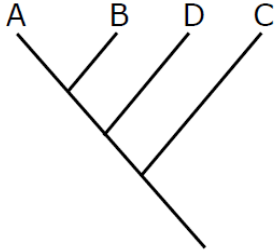


true species tree



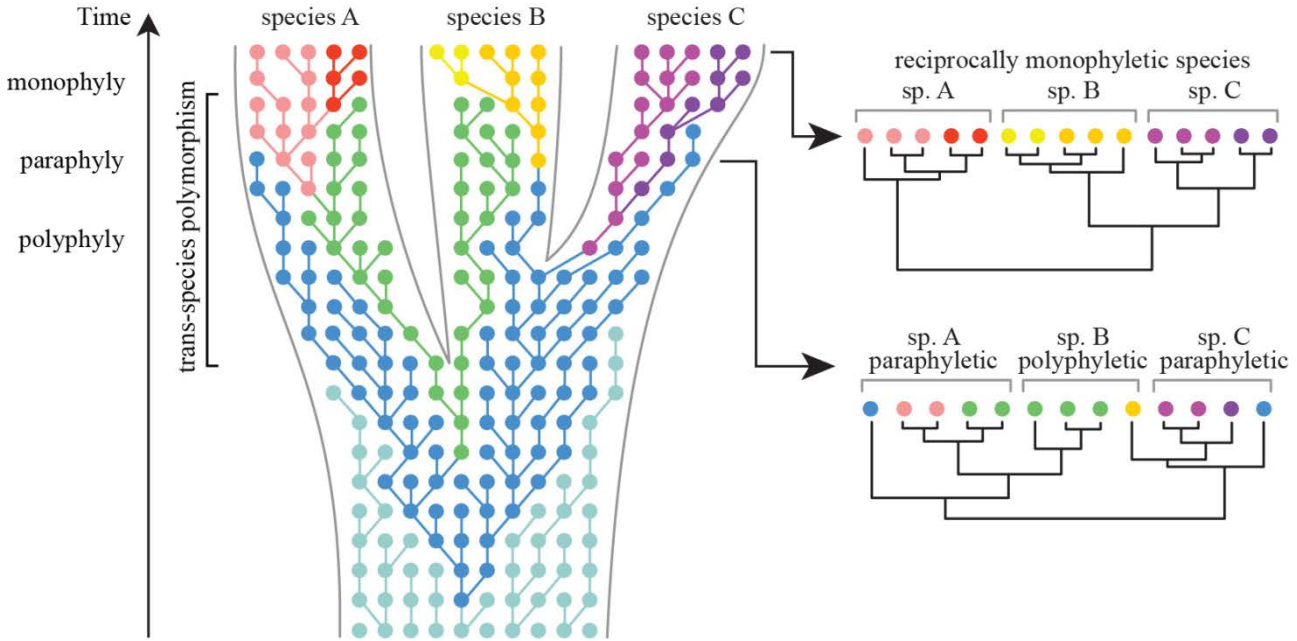


inferred species tree

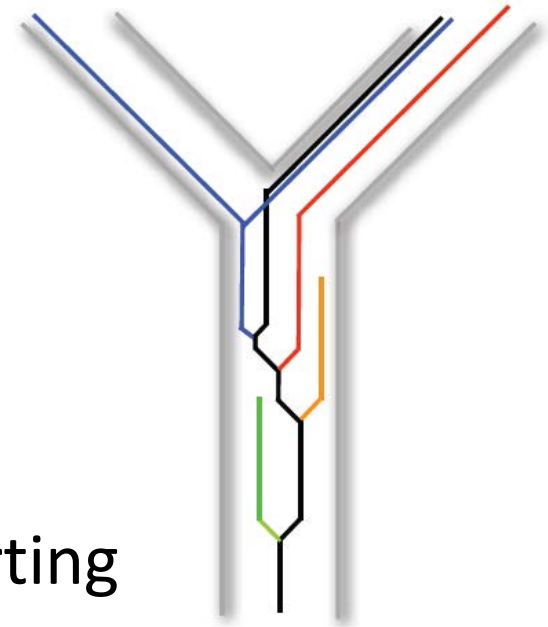


M. Popp, Oslo

Coalescence processes



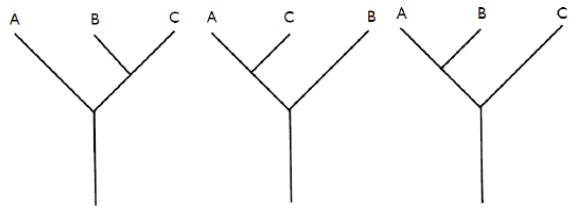
https://frederikleliaert.wordpress.com/green-algae/dna-based-species-delimitation-in-algae/

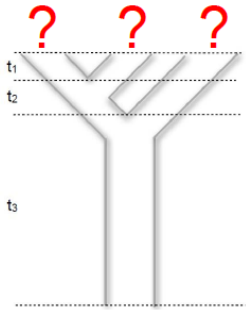


incomplete lineage sorting

M. Popp, Oslo

Species tree estimation





- **concatenation** (supermatrix) – good unless strong ILS
  - single partition model (e.g. MP)
  - multiple partitions model (ML or Bayesian)
- **consensual methods** using MP – minimizes deep coalescences (MDC)
- multispecies coalescence (all incongruences due to differences in coalescence processes, no hybridization)
  - **coestimation** of gene trees and species tree – *BEAST – Bayesian analysis (not applicable to large datasets)
  - **summary methods**
    - supertree methods – MRL (maximum representation using likelihood)
    - MP-EST – maximum likelihood estimation of rooted species tree
    - ASTRAL, ASTRID, STAR, STEAC – very fast and accurate
- Bayesian concordance analysis (BUCKy) – quartet-based Bayesian species tree estimation – uses concordance factor to build dominant history

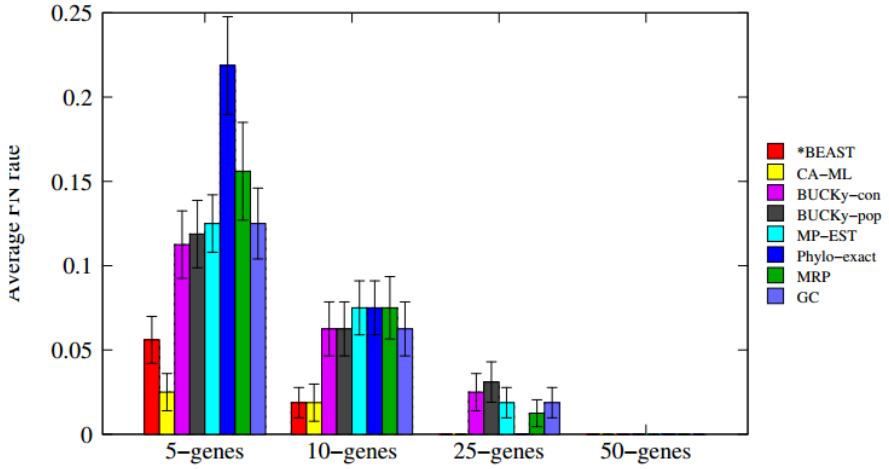
Summary methods

Species tree estimation

- supertree
  - MRP – maximum representation using parsimony
  - MRL – maximum representation using likelihood
- MP-EST – maximum pseudo-likelihood approach for estimating species trees
- STEAC – species tree estimation using average coalescence times
- STAR – species tree estimation using average ranks of coalescences
- ASTRAL – Accurate Species Tree Reconstruction ALgorithm
- ASTRID – Accurate Species TRees from Internode Distances (reimplementation of NJ_{st} method)

Methods comparison

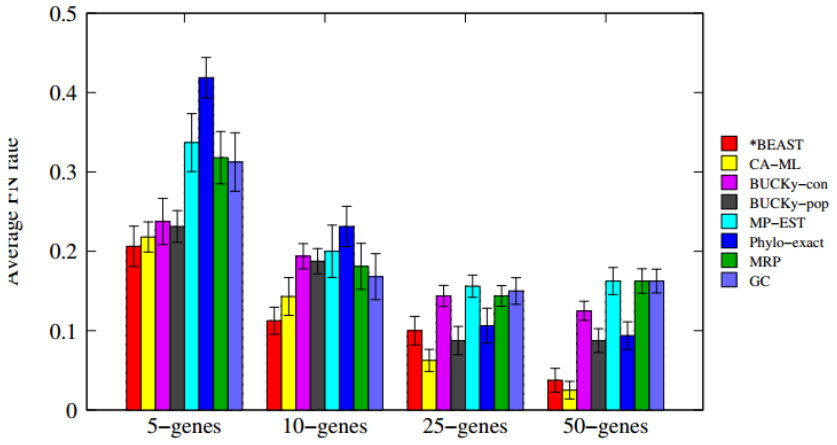
Results on 11-taxon datasets with weak ILS



*BEAST more accurate than summary methods (MP-EST, BUCKy, etc)
CA-ML: concatenated analysis) most accurate

Datasets from Chung and Ané, 2011
Bayzid & Warnow, Bioinformatics 2013

Results on 11-taxon datasets with strongILS

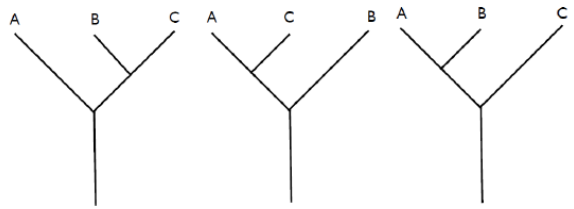


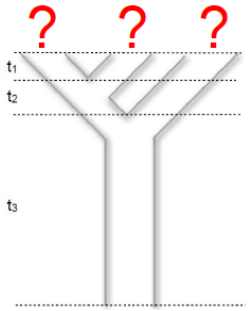
*BEAST more accurate than summary methods (MP-EST, BUCKy, etc)
CA-ML: (concatenated analysis) also very accurate

Datasets from Chung and Ané, 2011
Bayzid & Warnow, Bioinformatics 2013

T. Warnow, The University of Texas
https://www.cs.utexas.edu/users/tandy/394C-nov20-2013.pdf

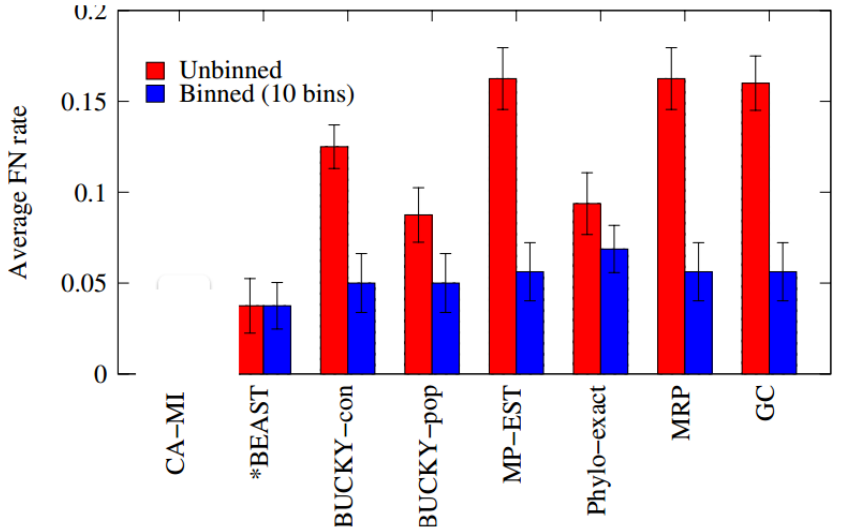
Species tree estimation





- wrong species tree if poor gene trees
  - shorter alignments usually give poorly supported trees
- improve gene trees
  - collapse unsupported branches
  - **binning** – assign gene to bins
    – create supergene alignments
    - **naïve binning** – random
    - **statistical binning**
      – no incompatibility among gene trees in the same set

11-taxon strongILS with 50 genes



T. Warnow, The University of Texas
https://www.cs.utexas.edu/users/tandy/394C-nov20-2013.pdf

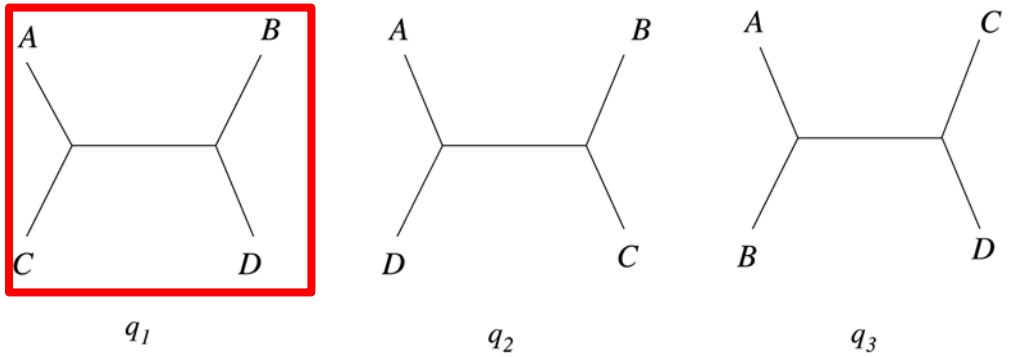
ASTRAL

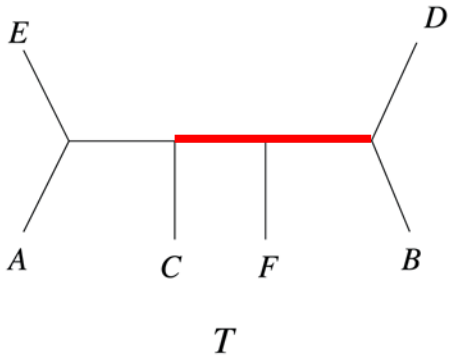
Accurate Species Tree Reconstruction ALgorithm

- unrooted gene trees
- species tree that agrees with the largest number of quartet trees induced by the set of gene trees
- weighting all three alternative quartet topologies according to their relative frequencies within gene trees
  - much more frequent topology – trees without this topology are penalized
  - similar frequencies (i.e., close to 0.33) – the quartet has little impact to optimization
- final species tree with
  - local posterior probability that the branch is in the species tree
  - the length of internal branches in coalescent units

Tree reconstruction from quartets

- quartet – unrooted tree over 4 taxa
- three possible quartets
- only one quartet *q* is consistent with final tree **T**





Reaz et al. (2015): *Accurate Phylogenetic Tree Reconstruction from Quartets: A Heuristic Approach*. PLoS ONE 9, e104008.

MRL

Maximum Representation with Likelihood

- supertree methods – estimates species tree on full taxon sets from sets of smaller trees (i.e., with missing species)
- encodes a set of gene trees by a **large randomized matrix**
- each edge (branch) in each gene tree
  - '0' for the taxa that are on one side of the edge
  - '1' for the taxa on the other side
  - '?' for all the remaining taxa (i.e., the ones that do not appear in the tree)
- MRL matrix is analyzed using heuristics for a symmetric 2-state Maximum Likelihood
  - in RAxML as 'BINCAT' model

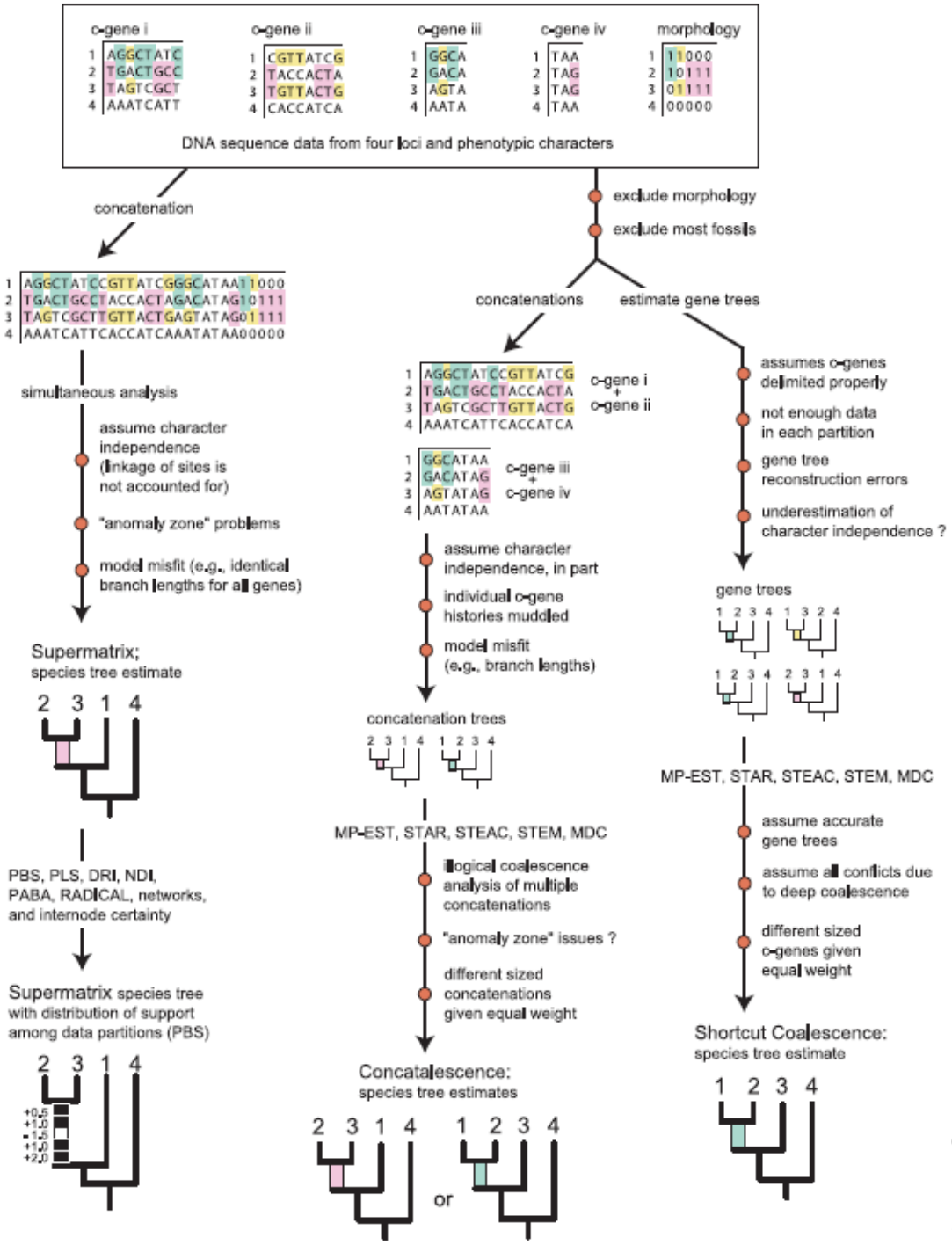
- similarly MRP – matrix analyzed with parsimony

Concatenation vs. coalescence

- concatenation
  - in favor: longer datasets allow for hidden support to appear
  - against: could be misleading under strong ILS

- coalescence (i.e., “shortcut coalescence” or summary methods)
  - in favor: addresses ILS
  - against:
    - short genes give poor gene trees (big problem!)
    - definition of coalescence-gene (segments with no internal recombining) debatable
    - concatenating coalescence-genes to longer alignments (“concatalescence”) not recommended?

see also:
Gatesy & Springer (2014): *Phylogenetic analysis at deep timescales: Unreliable gene trees, bypassed hidden support, and the coalescence/concatalescence conundrum*. Molecular Phylogenetics and Evolution 80: 231–266.



Gatesy & Springer. 2014. Molec. Phylog. Evol. 80: 231–266.

Filtering datasets

single-copy genes with good properties (no paralogs, low conflicting signal…) – filter out contaminants

- BLAST-based searches
- remove taxa with long branches
- remove poorly aligned regions

alignments

- length – longer better
- missing data – fewer better
- parsimony informative sites – more better
- information content

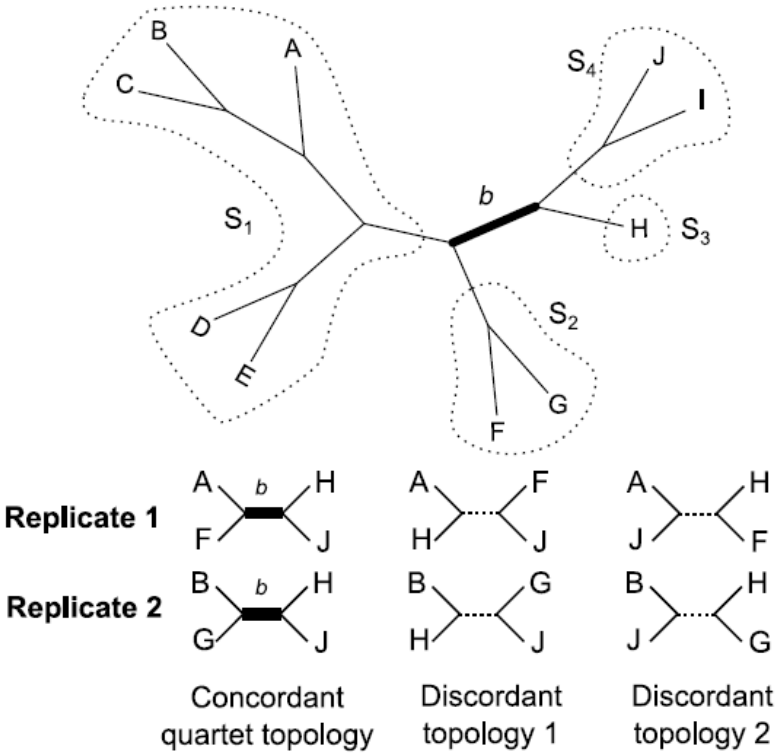
trees

- average bootstrap support – higher better
- average branch length – higher means faster gene
- saturation – correlation between p-distances and tree distance

Molloy & Warnow (2018): To include or not to include: the impact of gene filtering on species tree estimation methods. *Systematic Biology* 67: 285-303.
Herrando-Moraira et al. (2018): Exploring data processing strategies in NGS target enrichment to disentangle radiations in the tribe Cardueae (Compositae). *Molecular Phylogenetics and Evolution* 128: 69-87.

Quartet support

Replacement for bootstrap in phylogenomic studies…



Quartet Sampling Internal Node Scores = **0.52** / **0.91** / **0.95**

Quartet Concordance (QC)
How often is the concordant quartet inferred over both discordant quartets?
QC=1 → all concordant
QC=0 → equivocal conc./disc.
QC<0 → discordant > conc.

Quartet Differential (QD)
Are discordant #1 and #2 frequencies equal or skewed?
QD=1 → equal #1 and #2
QD=0.3 → skewed
QD=0 → all #1 or #2

Quartet Informativeness (QI)
What proportion of replicates were informative? (exceeded likelihood differential)
QI=1 → all informative
QI=0.3 → 30% informative
QI=0 → none informative

Quartet Sampling Terminal Node Scores = **(0.52)**

Quartet Fidelity (QF)
When this taxon is sampled, how often does it produce a concordant topology?
Examples:
QF=1 → all concordant
QF=0.1 → 10% concordant
QF=0 → none concordant

Pease et al. (2018): Quartet Sampling distinguishes lack of support from conflicting support in the green plant tree of life. *American Journal of Botany* 105(3): 385–403.

Phylotranscriptomic analysis of the origin and early diversification of land plants

Wickett et al., 2014, PNAS

- capstone paper from oneKP project
- transcriptomes from 92 streptophyte taxa + 11 genomes
- up to 852 nuclear genes, ~1,700,000 sites
- 69 analyses
  - missing data filtering
  - supermatrix, supertree, coalescence-based
  - ML, Bayesian
  - partitioned/unpartitioned
  - amino acids, DNA

Taxonomic concepts

- Streptophytes – Klebsormidiales, Coleochaetales…, Charales, Zygnematophyceae +
- Embryophytes (land plants) – Anthocerotophyta (hornworts), Marchantiophyta (liverworts), Bryophyta (mosses) +
- Tracheophytes (vascular plants) – Lycopodiophyta(lycophytes) +
- Euphyllophytes – monilophytes (ferns) +
- Spermatophytes (seed plants) – Gymnosperms +
- Angiosperms (flowering plants) – ANA grade, monocots, magnoliids, eudicots

Introduction

- origin of embryophytes (land plants) – Ordovician (480 Mya)
- innovations – parental protection for embryo, alternation of generations (diploid sporophyte, haploid gametophyte)
- changes in global carbon cycle
- forming terrestrial ecosystems
- series of rapid radiations – most diverse group of extant plants

- main questions
  - which green algae lineage is most closely related to embryophytes?
  - what is the branching order among the main embryophyte lineages?

Previous studies

- streptophytes monophyletic, but…
- branching order relative to embryophytes uncertain
- shared characters among embryophytes, Charales, Coleochaetales
  - oogamous sexual reproduction
  - apical growth with branching
  - presence of plasmodesmata in gametophyte
  - phragmoplast (microtubulles and microfilaments directing formation of cell plate during cytokinesis)
- different relationships recovered
  - Charales sister to embryophyta
  - Coleochaetales/Zygnematophyceae sister to embryophyta
- different relationships of bryophytes, esp. position of hornworts
- position of Gnetales (*Gnetum*, *Welwitschia*, *Ephedra*) within gymnosperms

Methods

- 1KP consortium – transcriptomes
- 2x75- or 2x90-bp reads assembled with SOAPdenovo

- proteins from 25 sequenced plant genomes clustered to gene families (OrthoMCL)
- single-copy families identified, aligned (MAFFT), making profile database (HMMER3)
- transcriptomes translated to AA and searched against 25 genome profiles – most transcript sorted into a single family
- transcriptomes aligned and consensus sequence created
- if the consensus contained more than 5% ambiguities, the taxon/gene combination was excluded (duplication assumed)

Phylogenetic analyses

- 852 gene family files aligned with SATé – both AA and DNA
- RAxML gene trees with 200 bootstrap replicates
  - AA alignments (JTT model)
  - DNA alignments (GTR)
  - codon alignments (in-frame DNA)
  - codon alignments with 3rd position removed
- supermatrix (concatenation) – filtering
  - genes with less than 50% of taxa removed
  - sites with more than 50% of missing characters removed
  - genes not including *Chara* removed
  - taxa on very long branches removed
  - extensive trimming (blastp- and branch-length-based, GBLOCKS to remove poorly aligned positions)

Phylogenetic analyses

- ML supermatrix – RAxML (GTR for DNA, JTTF for AA), 100 bootstrap
  - unpartitioned
  - partitioned (for codon K-means clustering method used)
- PhyloBayes supermatrix
- coalescent-based analysis (ASTRAL) + multilocus bootstrap
  - all gene trees
  - only gene trees with more than 50% of taxa
  - gene trees after removing sequences with more than 66% gaps
  - gene trees after taxa on long branches removed
  - calculated conflict between species tree and gene trees for each branch
- supertree analysis (Superfine-MRP)

Results

- sequence alignments estimated for 9,610 gene families
- 852 families including at most one gene copy (from at least 24 of the 25 sequenced genomes)
- concatenated untrimmed matrix – 1,701,170 aligned sites
- 69 analyses in total – results highly concordant with ML tree based on 1st and 2nd codon positions
- 3rd codon position – large variation in GC content could lead to model misspecification

Streptophytic algae and land plants

- Streptophyta monophyletic
- Zygnematophyceae strongly supported as sister lineage of embryophytes – both supermatrix and ASTRAL analyses

- many gene trees with not strong support for hypotheses, small proportion of trees did exhibit well-supported conflict – this is probably due to incomplete lineage sorting of ancestral variation

- phragmoplast – secondary loss in most Zygnematophyceae

Bryophyte relationships

- monophyly of each lineage supported
- liverworts are NOT sister to vascular plants

- 3 alternative hypothesis supported:
- bryophytes monophyletic in ASTRAL and supertree analyses
  - mosses and liverworts monophyletic
- hornworts and moss+liverwort clade successively sister to vascular plants in supertree analysis
  - consistent with morphology and development (e.g., pyrenoid shared by hornworts and streptohytic algae)
- hornworts sister to vascular plants
  - consistent with similarity of gametangia development in hornworts to antheridial/archegonial development in monilophytes

Monilophyte and Lycophyte

- lycophytes and monilophytes are successively sister lineages to the seed plants
- agreement with previous phylogenetic analyses

- resolution of backbone phylogeny of ferns is problematic

- instability in the placement of *Equisetum*

Gymnosperm relationships

- strong monophyly
- Gnetales sister to all other lineages only in analyses with all three codon positions
- Gnetales sister to Coniferales – “Gnetifer” hypothesis
  - ASTRAL and supertree analyses
- Gnetales within Coniferales (sister to Pinaceae) – “Gnepine”
  - in supermatrix analyses
  - consistent with previous results
- rapid diversification among Gnetales and two conifer lineages
  - ILS – misleading supermatrix analyses

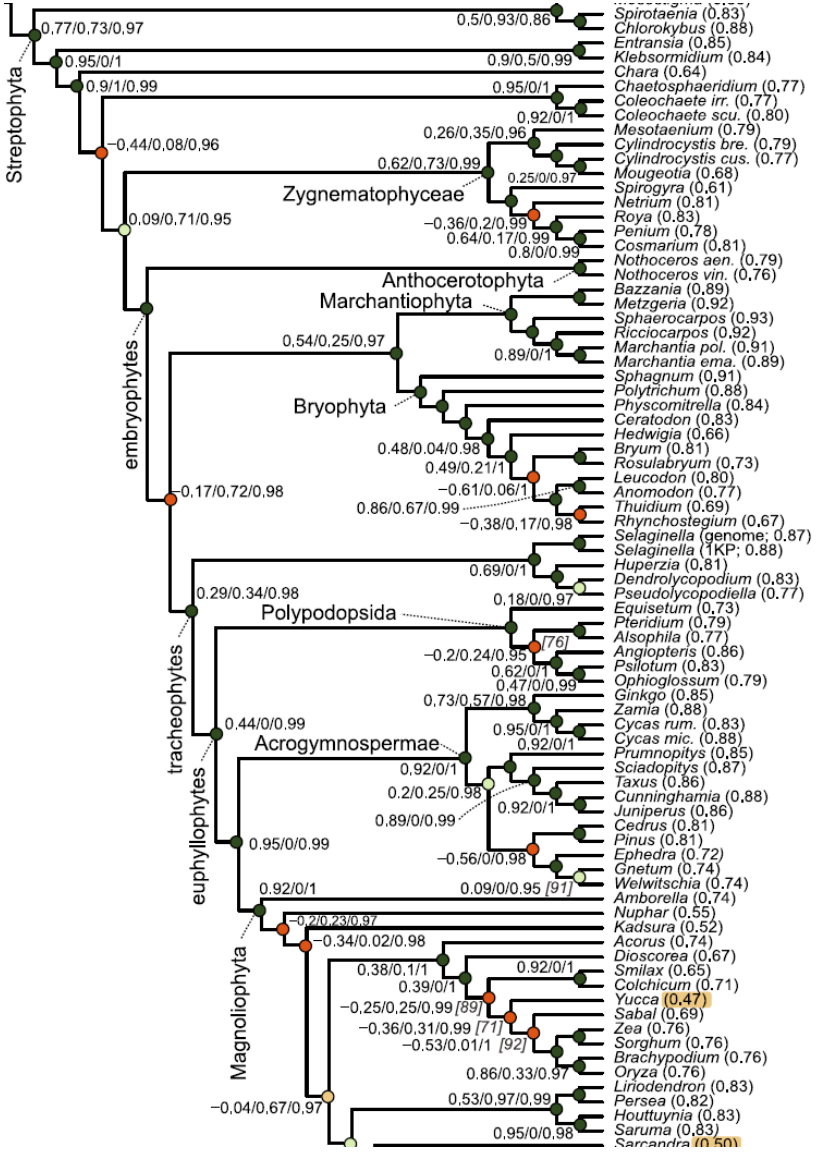
Angiosperm relationships

- rapid diversification of flowering plant lineages (Darwin's "abominable mystery" – resolution of branching remains controversial
- ANA (*Amborella*-Nymphaeales-Austrobaileyales) grade basal
  - *Amborella* as sister to all other angiosperms
  - Nympheaeles and Austrobaileyales successive sister lineages
- monocots sister to all other
- only PhyloBayes analysis of AA placed magnoliid+Chloranthales sister to eudicot+monocots

- variations in relationships due to
  - model misspecification (simplification)
  - ILS
- increased taxon sampling necessary

Quartet support

Replacement for bootstrap in phylogenomic studies…



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