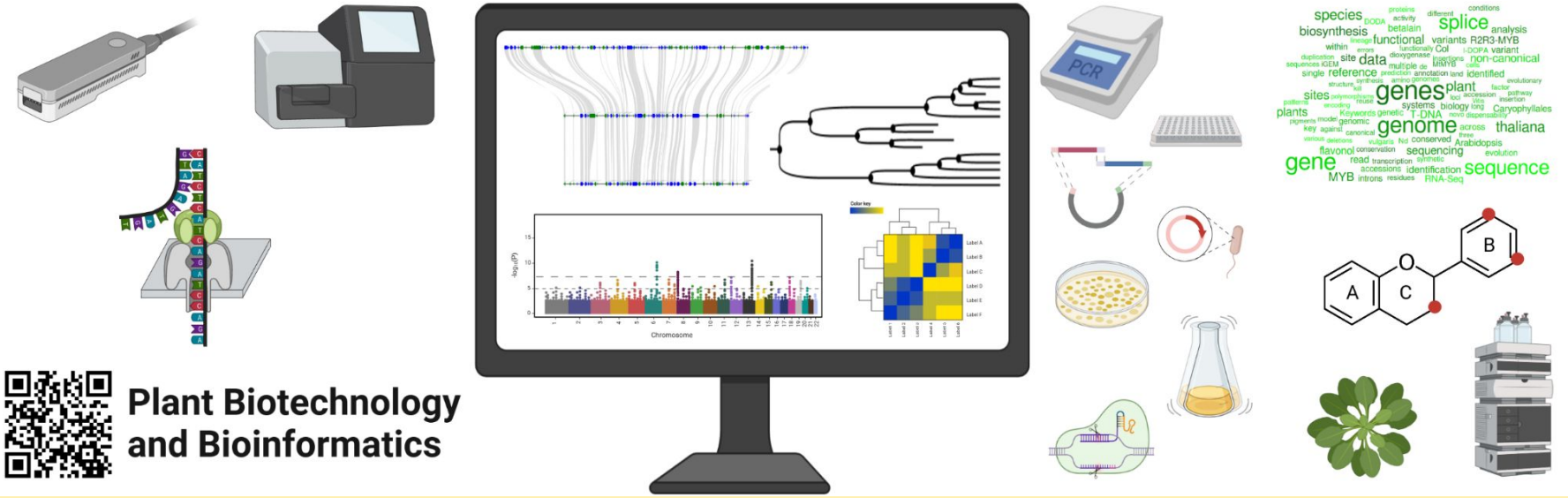
Technische Universität Braunschweig



Plant Biotechnology and Bioinformatics

Phylogenetic Analyses

Prof. Dr. Boas Pucker (Plant Biotechnology and Bioinformatics)

Availability of slides

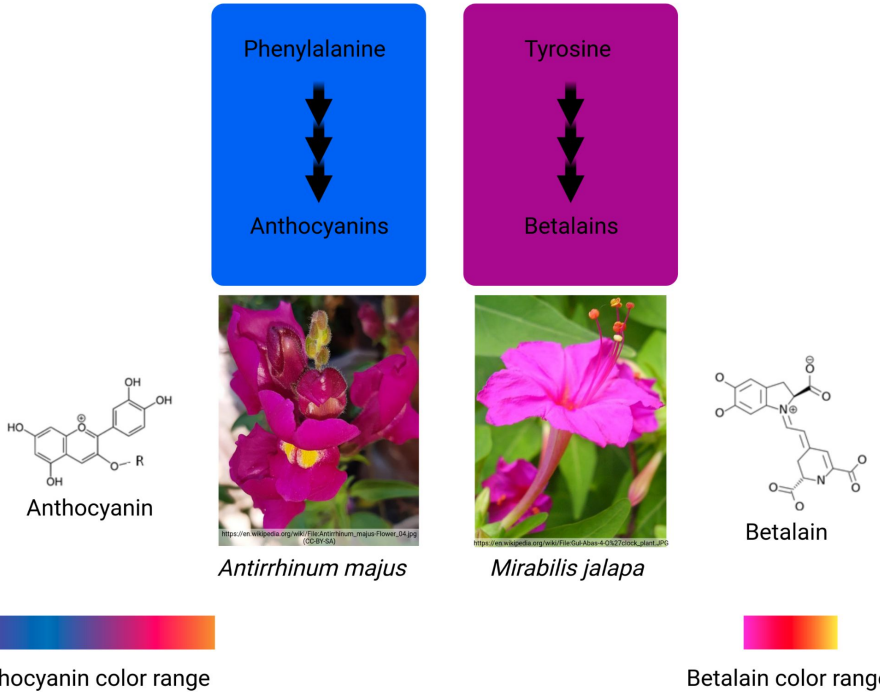
- All materials are freely available (CC BY) - after the lectures:
  - StudIP: Lecture: Grundlagen der Biochemie und Bioinformatik der Pflanzen (Bio-MB 09)
  - GitHub: https://github.com/bpucker/teaching

- Questions: Feel free to ask at any time

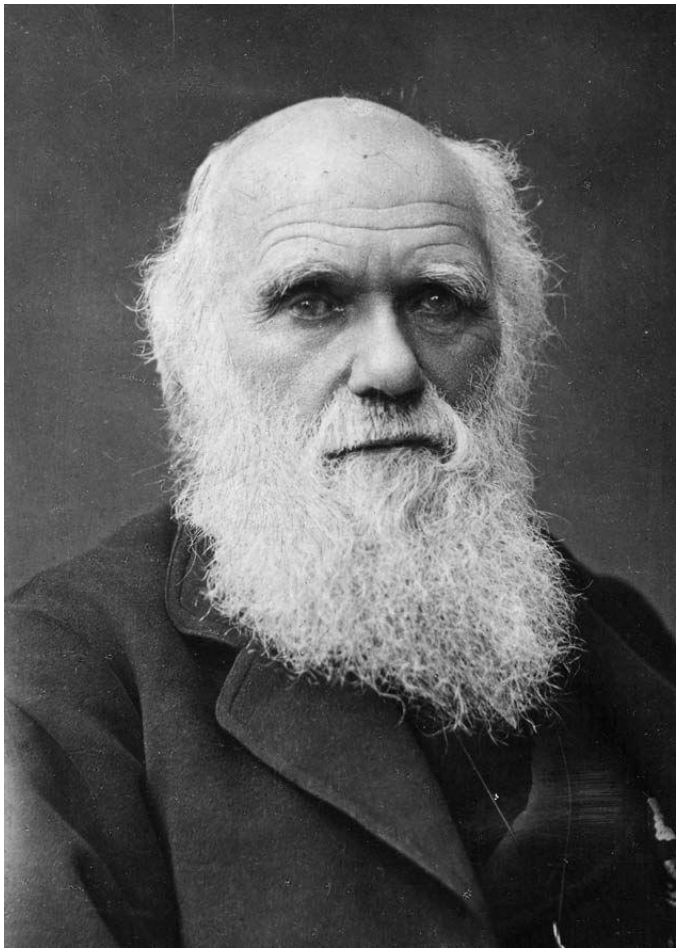
- Feedback, comments, or questions: b.pucker[a]tu-bs.de

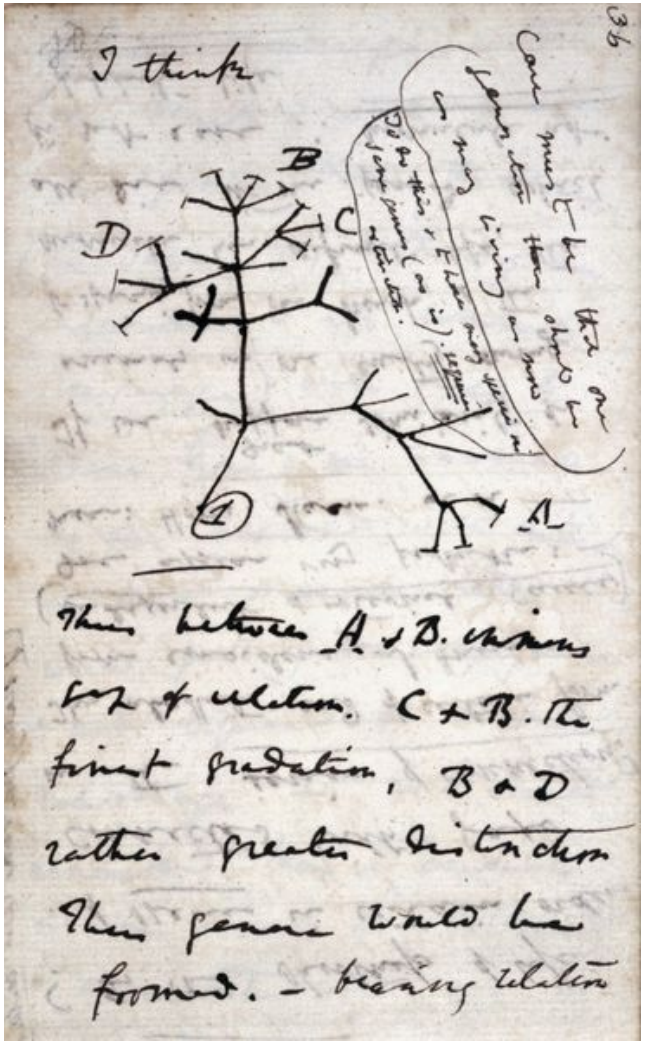
Theodosius Dobzhansky (1900-1975)

“Nothing in biology makes sense except in the light of evolution”



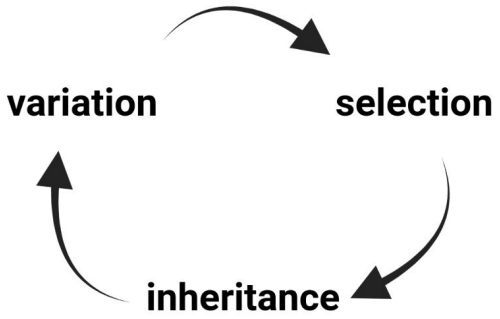
Charles Darwin (1809-1882)





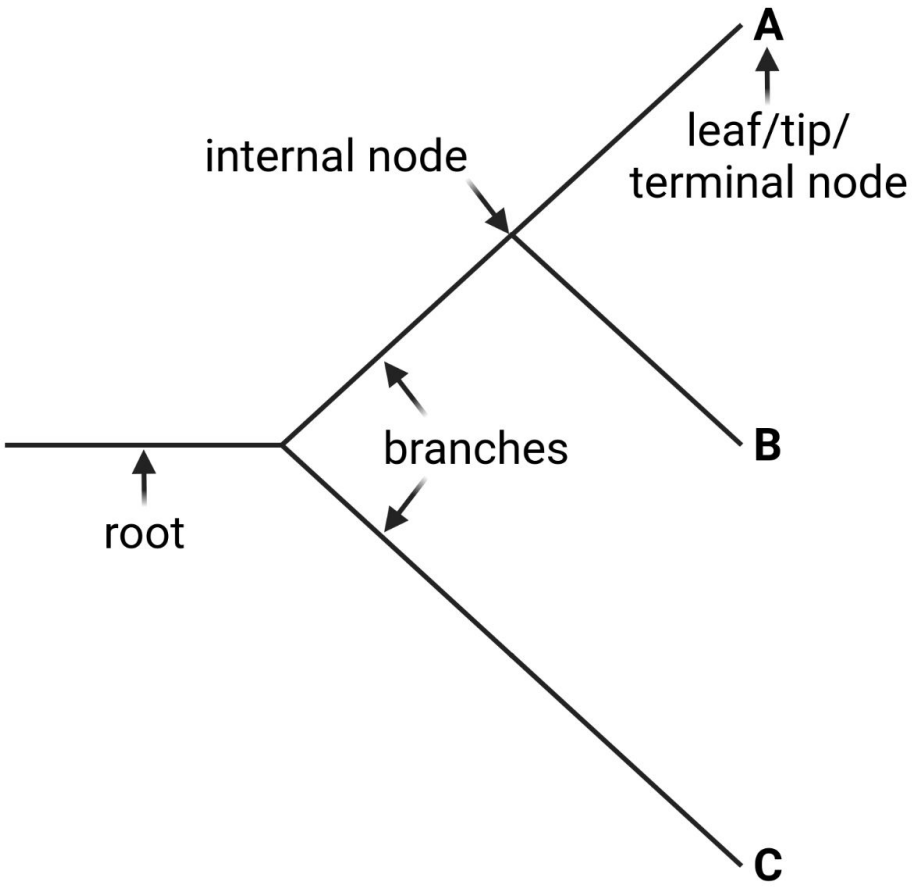
Basic evolution concept

- Selection is performed by nature (‘theory of natural selection’)
- Selection requires (1) variation to act upon and (2) more offspring than sustainable
- Inheritance determines the properties of individuals (phenotype)

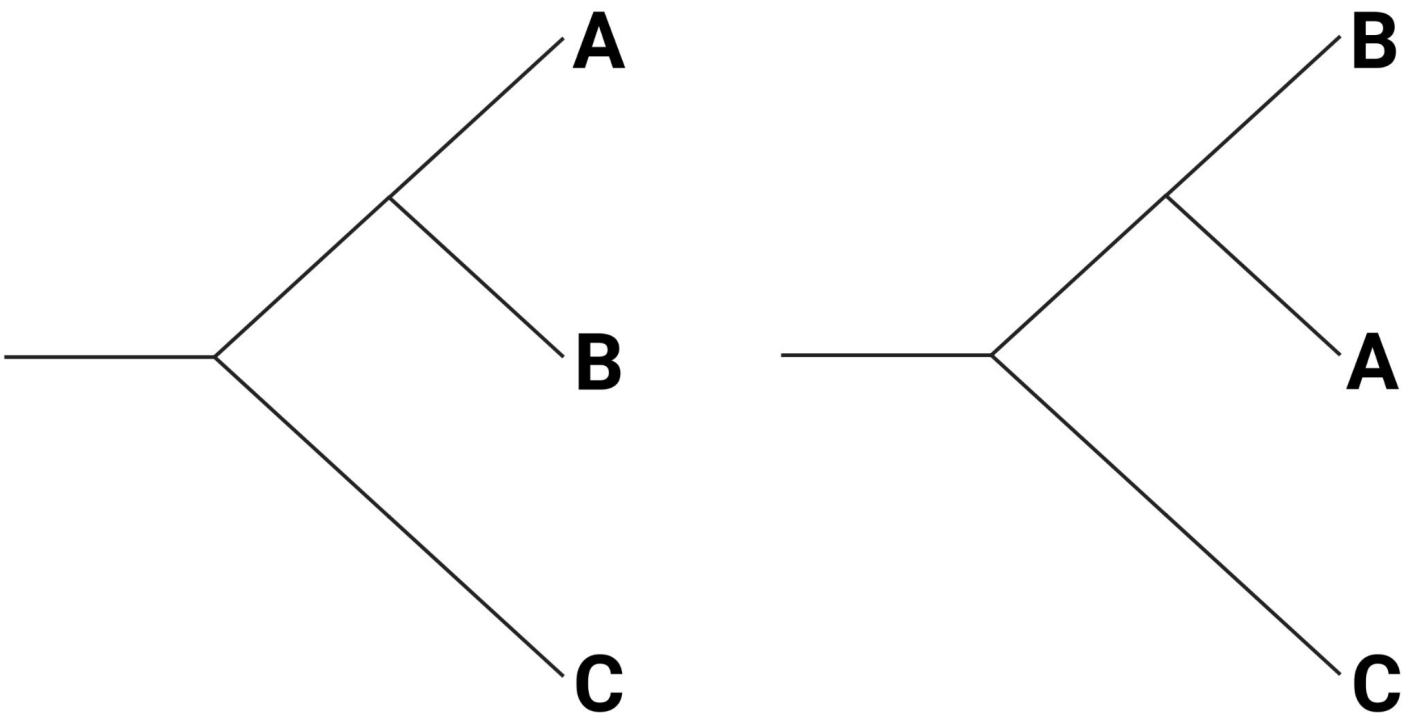


Richard Dawkins: The Selfish Gene; The Extended Phenotype

Elements of a phylogenetic tree

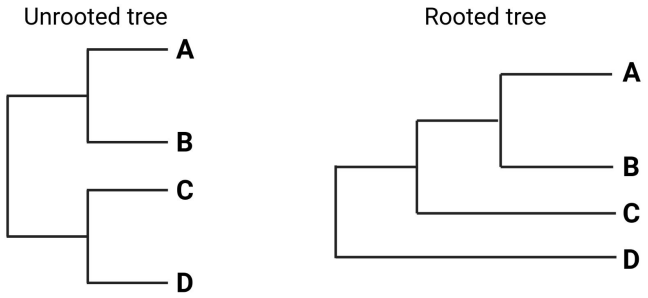


Tree topology and rotation

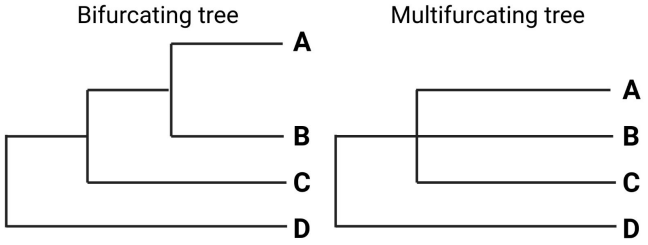


Types of trees

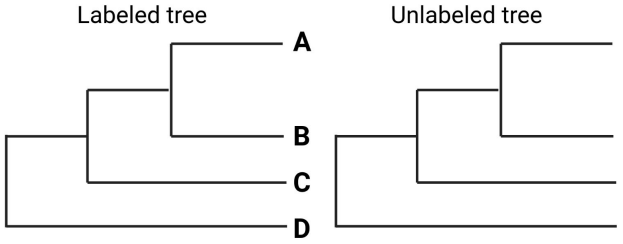
- Rooted vs. unrooted tree



- Bifurcating vs. multifurcating tree

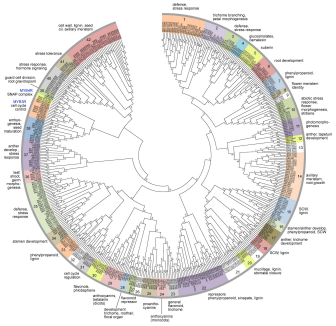


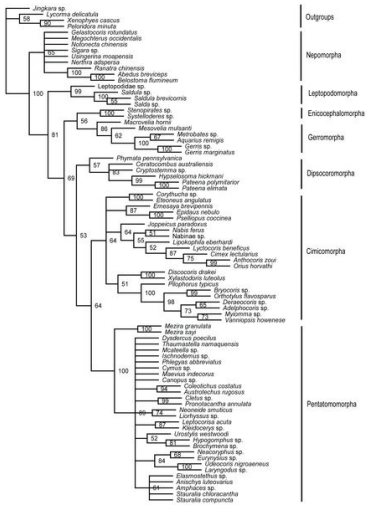
- Labeled vs. unlabeled trees

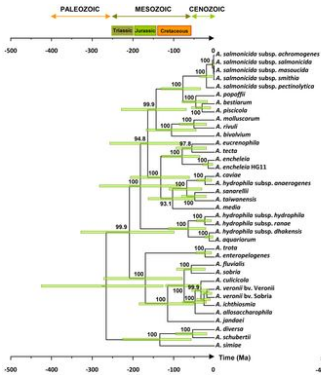


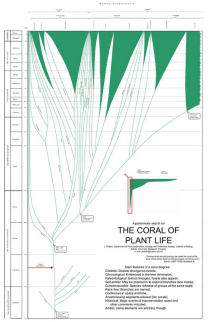
Types of special trees

- Cladogram: only represent the branching pattern without branch lengths
- Phylogram: branch lengths indicate number of changes
- Chronogram: branch lengths indicate time
- Spindle diagram: shows taxonomic diversity over time





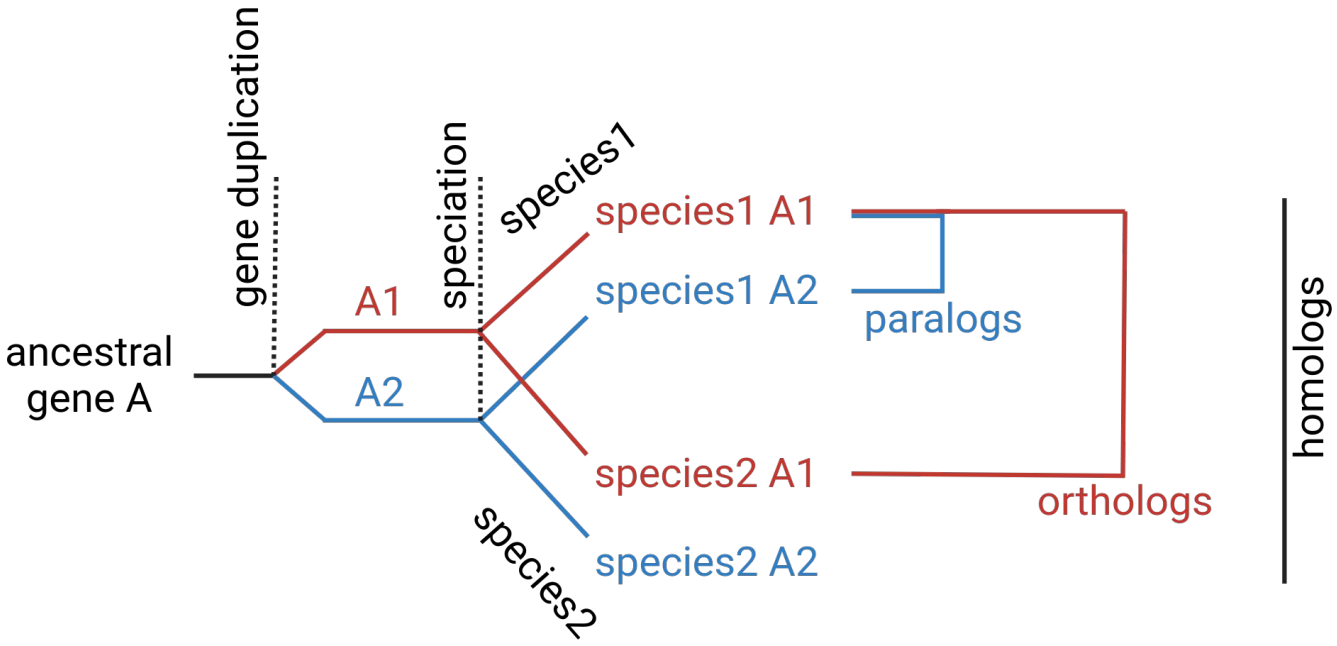




Pucker et al., 2020: 10.1371/journal.pone.0239275
Li et al., 2012: 10.1371/journal.pone.0032152
Loren et al., 2014: 10.1371/journal.pone.0088805
Podani et al., 2020: 10.5586/asbp.8937

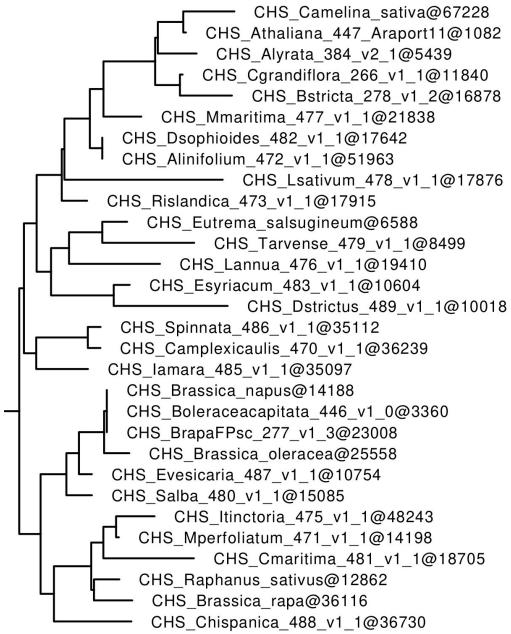
Homologs, orthologs, and paralogs

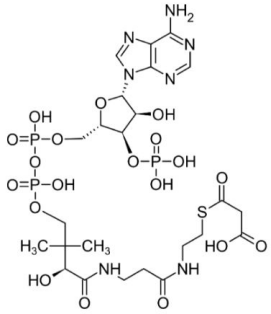
- Homolog = genes with common ancestors
- Ortholog = same gene in different species
- Paralog = gene copies in one species



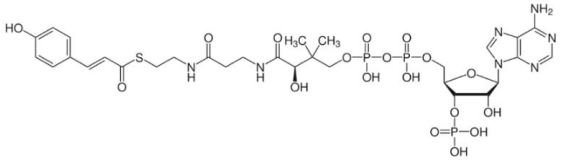
Example: orthologs

- CHS in different species has the same function
- Reaction is initial step of the flavonoid biosynthesis
- Flavonoid biosynthesis is conserved across plant species

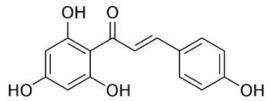




3x malonyl-CoA



4-coumaroyl-CoA



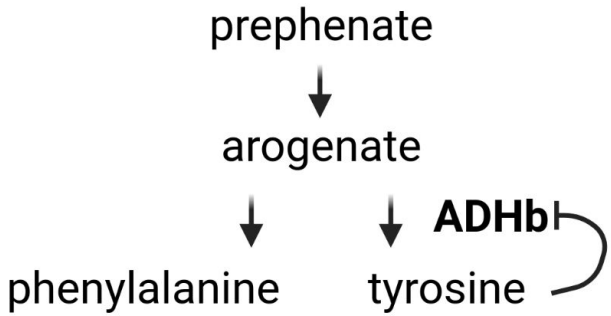
naringenin chalcone

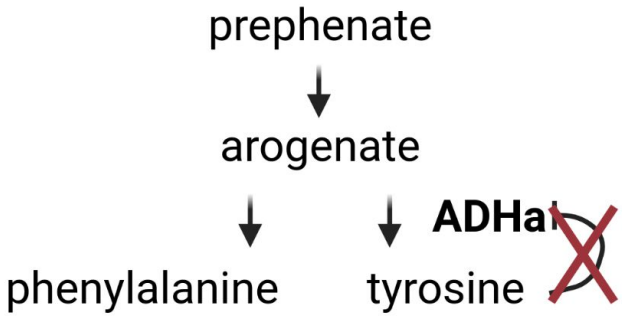
(4x CoA + 3x CO_2)

Pucker et al., 2021: 10.1186/s12870-021-03080-9

Example: paralogs

- ADH was duplicated in Caryophyllales
  - ADHa = feedback-resistance enzyme
  - ADHb = feedback-inhibited enzyme

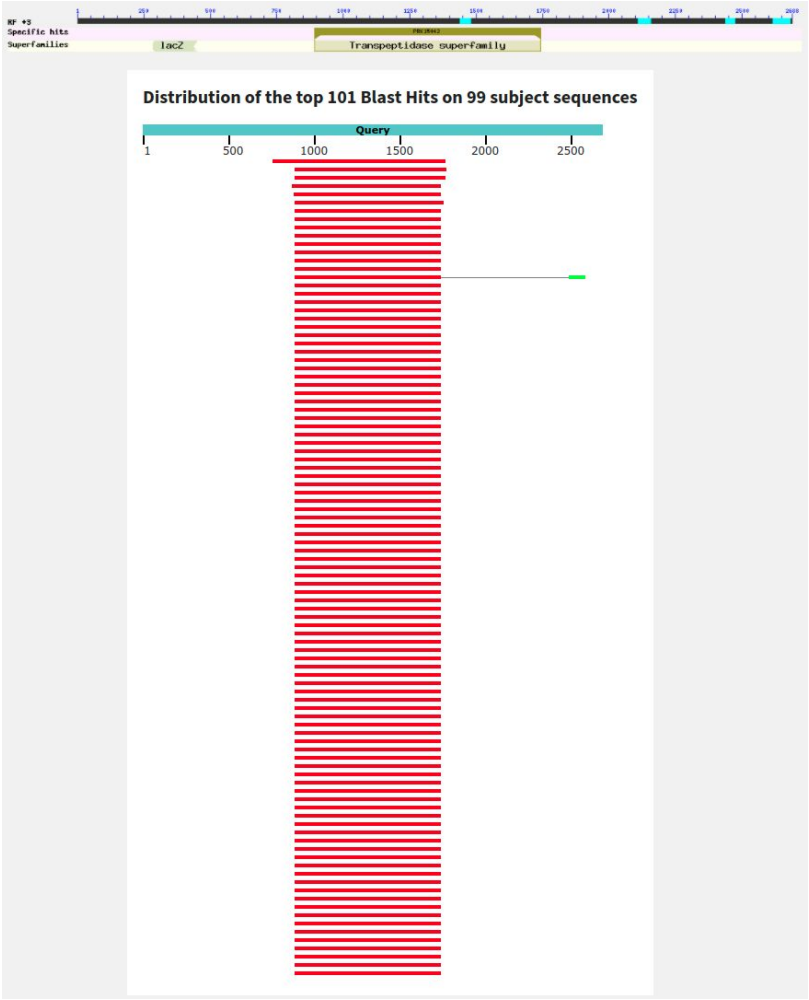




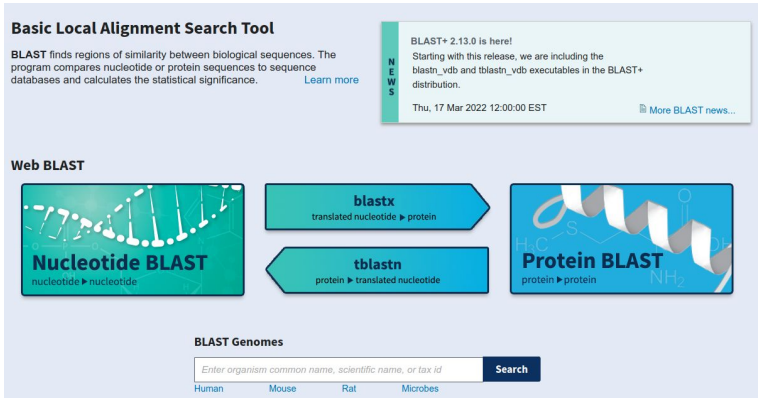
https://commons.wikimedia.org/wiki/File:Zuckerr%C3%BCbe.jpg (CC BY-SA 3.0)
Lopez-Nieves et al., 2017: 10.1111/nph.14822

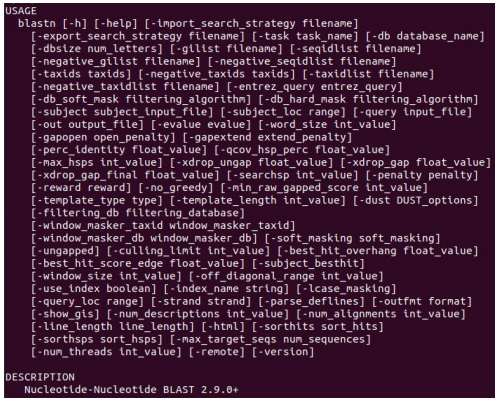
BLAST - simple sequence searches

- BLAST = Basic Local Alignment Search Tool
- Identification of short stretches of sequence similarity
- Quick search against a large database
- Sequence similarity suggest homology i.e. common ancestors and similar function



NCBI website vs. local BLAST





NCBI website	Local BLAST
Convenient to use	Requires some command line knowledge
Requires no local computational resources	Computational resources needed
Transfer of query sequence	Query kept secret
Large databases available	Download of databases required

BLAST alternatives

- HMMER: http://hmmer.org/
  - Dedicated to the identification of specific domains

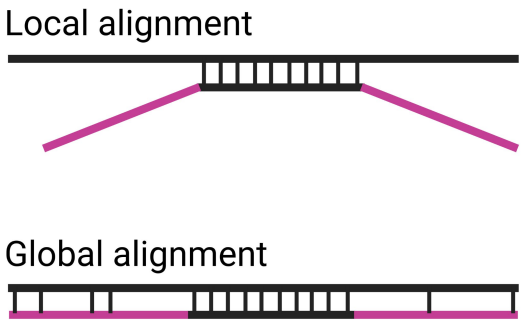
- DIAMOND: https://github.com/bbuchfink/diamond
  - Much faster, but higher memory requirements

http://hmmer.org/
Mistry, 2013: 10.1093/nar/gkt263
https://github.com/bbuchfink/diamond
Buchfink et al., 2021: 10.1038/s41592-021-01101-x

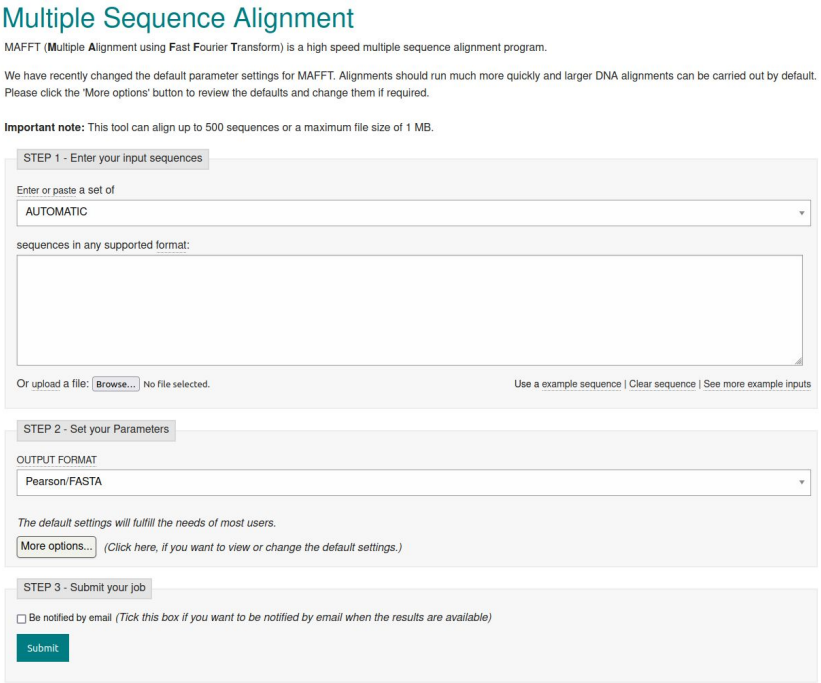
Local vs. global alignment

- Local alignment highlights small stretches with high similarity
  - random hit possible
  - better for strong sequence length differences

- Global alignment only works by overall high similarity
  - Full length similarity supports similar function



MAFFT: online vs. local




```
--------------------------------------------------------------------------------
  MAFFT v7.453 (2019/Nov/8)
  https://mafft.cbrc.jp/alignment/software/
  MBE 30:772-780 (2013), NAR 30:3059-3066 (2002)
--------------------------------------------------------------------------------
High speed:
  % mafft in > out
  % mafft --retree 1 in > out (fast)

High accuracy (for <~200 sequences x <~2,000 aa/nt):
  % mafft --maxiterate 1000 --localpair  in > out (% linsi in > out is also ok)
  % mafft --maxiterate 1000 --genafpair  in > out (% einsi in > out)
  % mafft --maxiterate 1000 --globalpair in > out (% ginsi in > out)

If unsure which option to use:
  % mafft --auto in > out

--op # :         Gap opening penalty, default: 1.53
--ep # :         Offset (works like gap extension penalty), default: 0.0
--maxiterate # : Maximum number of iterative refinement, default: 0
--clustalout :   Output: clustal format, default: fasta
--reorder :      Outorder: aligned, default: input order
--quiet :        Do not report progress
--thread # :     Number of threads (if unsure, --thread -1)
--dash :         Add structural information (Rozewicki et al, submitted)
```

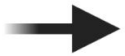
https://www.ebi.ac.uk/Tools/msa/mafft/

Alignment construction

- Letters representing amino acid residues are arranged to highlight similarities
- Gaps (-) are inserted to show differences

Input FASTA file with sequences

```
>seq1
MDTEKYMEKWIDQGHALFPEEDQ
>seq2
MDTDIKYSEKWIQGHSLFPDDQ
>seq3
METEKYMEKWIQGHSIFPEDQ
>seq4
MDTEIKYMEKWIQGHALFPDDQP
```



Alignment of sequences

```
seq1    MDTE-KYMEKWIDQGHALFPEEDQ-
seq2    MDTDIKYSEKWI-QGHSLFPD-DQ-
seq3    METE-KYMEKWI-QGHSIFPE-DQ-
seq4    MDTEIKYMEKWI-QGHALFPD-DQP
```

Alignment formats

CLUSTAL

```
AthCHS          MVMAGASSLDEIRQAQRADGPAGILAIGTANPENHVLQAEYPDYYFRITNSEHMTDLKEK
BvuCHS          M---ATPSVQEIRDAQRSNGPATILAIGTANPANEMYQAEYPDFYFRVTKSEHMSELKQK
                *   .:.*::***:***::*** ********* *.: ******:***:*:****::**:*

AthCHS          FKRMCDKSTIRKRHMHLTEEFLKENPHMCAYMAPSLDTRQDIVVVEVPKLGKEAAVKAIK
BvuCHS          FKRMCDKSMIKKRYMHVTQELLEENPHMCDYNASSLNTRQDILATEVPKLGKEAAVKAIK
                ******** *:**:**:*:*:*:****** * *.**:*****:..***************

AthCHS          EWGQPKSKITHVVFCTTSGVDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRIAK
BvuCHS          EWGQPRSKITHVIFCTTSGVDMPGADYQLTKLLGLRPSVKRFMLYQQGCYAGGTVLRLAK
                *****:******:*****************************:*:*****:*******:**

AthCHS          DLAENNRGARVLVVCSEITAVTFRGPSDTHLDSLVGQALFSDGAAALIVGSDPDTSVGEK
BvuCHS          DIAENNRGARVLVVCAEITVICFRGPTETHLDSMIGQALFGDGAGAVIVGADLDESI-ER
                *:**************:***.: ****::*****::*****.***.*:***:* * *: *:

AthCHS          PIFEMVSAAQTILPDSDGAIDGHLREVGLTFHLLKDVPGLISKNIVKSLDEAFKPLGISD
BvuCHS          PIFQLVWAAQTILPDSEGAIDGHLREVGLAFHLLKDVPGLISKNIEKALVEAFKPIGIDD
                ***::* *********:*************** *:* *****:**.*

AthCHS          WNSLFWIAHPGGPAILDQVEIKLGLKEEKMRATRHVLSEYGNMSSACVLFILDEMRRKSA
BvuCHS          WNSIFWAAHPGGPAILDQVESKLGLKQDKLSTTRHVLSEFGNMSSACVLFILDEMRKRSM
                ***:** ************* *****::*: :*******:*****************::*

AthCHS          KDGVATTGEGLEWGVLFGFGPGLTVETVVLHSVPL--
BvuCHS          KEGMATTGEGLEWGVLFGFGPGLTVETVMLHSVPIAN
                *:*:*************************:*****:
```

FASTA

```
>AthCHS
MVMAGASSLDEIRQAQRADGPAGILAIGTANPENHVLQAEYPDYYFRITNSEHMTDLKEK
FKRMCDKSTIRKRHMHLTEEFLKENPHMCAYMAPSLDTRQDIVVVEVPKLGKEAAVKAIK
EWGQPKSKITHVVFCTTSGVDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRIAK
DLAENNRGARVLVVCSEITAVTFRGPSDTHLDSLVGQALFSDGAAALIVGSDPDTSVGEK
PIFEMVSAAQTILPDSDGAIDGHLREVGLTFHLLKDVPGLISKNIVKSLDEAFKPLGISD
WNSLFWIAHPGGPAILDQVEIKLGLKEEKMRATRHVLSEYGNMSSACVLFILDEMRRKSA
KDGVATTGEGLEWGVLFGFGPGLTVETVVLHSVPL--
>BvuCHS
M---ATPSVQEIRDAQRSNGPATILAIGTANPANEMYQAEYPDFYFRVTKSEHMSELKQK
FKRMCDKSMIKKRYMHVTQELLEENPHMCDYNASSLNTRQDILATEVPKLGKEAAVKAIK
EWGQPRSKITHVIFCTTSGVDMPGADYQLTKLLGLRPSVKRFMLYQQGCYAGGTVLRLAK
DIAENNRGARVLVVCAEITVICFRGPTETHLDSMIGQALFGDGAGAVIVGADLDESI-ER
PIFQLVWAAQTILPDSEGAIDGHLREVGLAFHLLKDVPGLISKNIEKALVEAFKPIGIDD
WNSIFWAAHPGGPAILDQVESKLGLKQDKLSTTRHVLSEFGNMSSACVLFILDEMRKRSM
KEGMATTGEGLEWGVLFGFGPGLTVETVMLHSVPIAN
```

Alignment trimming

- Removal of alignment columns with low abundance
- Columns with low abundance are not informative
- Reduced alignment lengths makes tree construction easier

Alignment of sequences

```
seq1    MDTE-KYMEKWIDQGHALFPEEDQ-
seq2    MDTDIKYSEKWI-QGHSLFPD-DQ-
seq3    METE-KYMEKWI-QGHSIFPE-DQ-
seq4    MDTEIKYMEKWI-QGHALFPD-DQP
```

Trimmed alignment of sequences

```
seq1    MDTE-KYMEKWIQGHALFPEDQ
seq2    MDTDIKYSEKWIQGHSLFPDDQ
seq3    METE-KYMEKWIQGHSIFPEDQ
seq4    MDTEIKYMEKWIQGHALFPDDQ
```

Construction of a phylogenetic tree

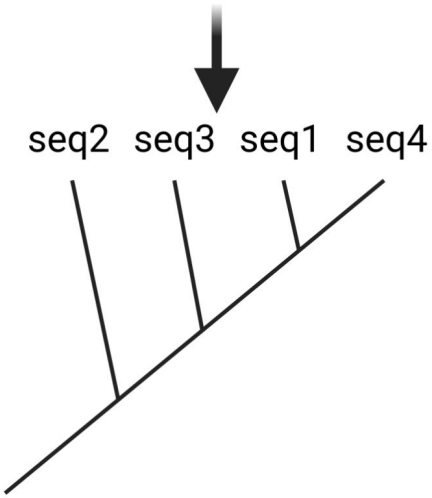
- Construction of phylogenetic trees is based on sequence alignments
- Similar sequences (recent shared ancestors) are grouped

Trimmed alignment of sequences

```
seq1    MDTE-KYMEKWIQGHALFPEDQ
seq2    MDTDIKYSEKWIQGHSLFPDDQ
seq3    METE-KYMEKWIQGHSIFPEDQ
seq4    MDTEIKYMEKWIQGHALFPDDQ
```

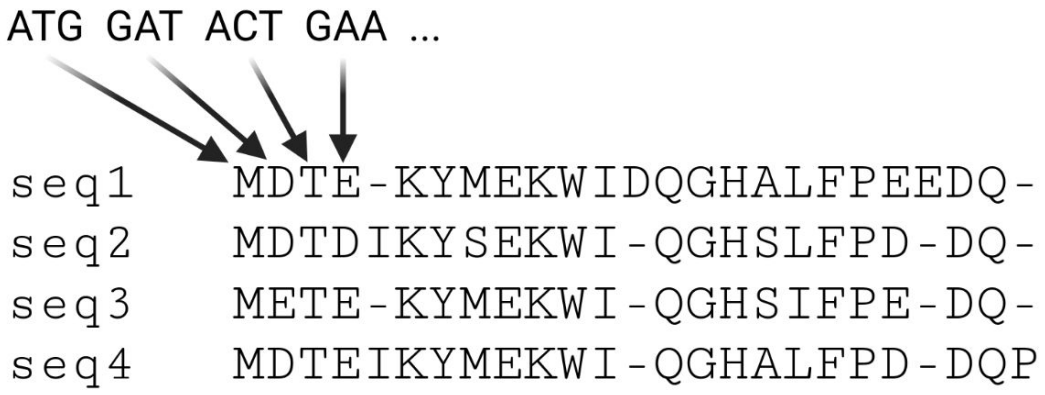
Distance calculation

	seq1	seq2	seq3	seq4
seq1	.	5	3	2
seq2	.	.	6	3
seq3	.	.	.	5
seq4	.	.	.	.



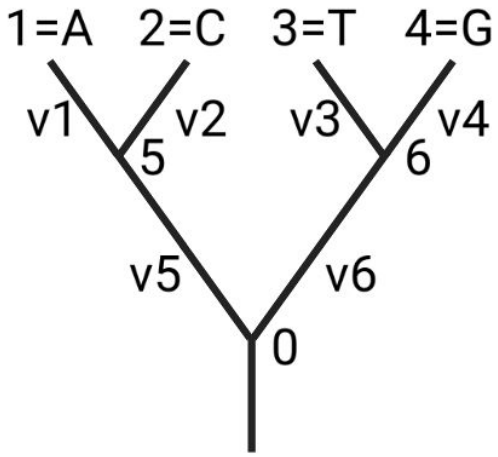
Substituting amino acids with codons

- Phylogenetic signal is boosted by substitution of amino acids by codons
- More positions that can show variation
- Alignment can still be based on amino acid residues (avoids splitting codons)



Maximum Likelihood (ML) trees

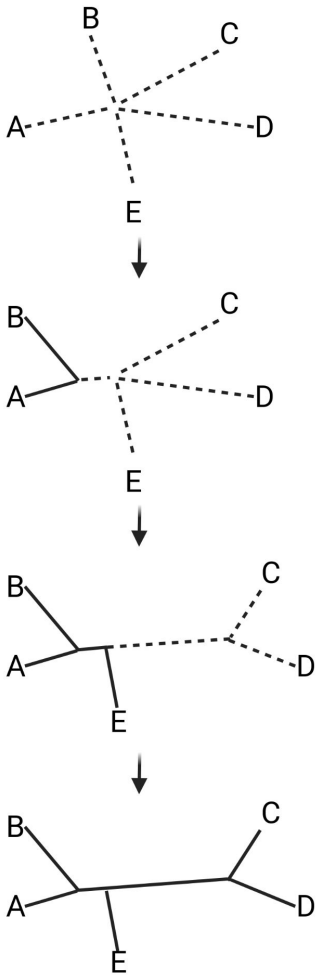
- Finding the tree with highest likelihood to explain the observed sequences
- Number of possible trees depends on number of sequences
- Often impossible to explore all possible trees (very computationally demanding)
- Tool: RAxML-NG



Cho, 2012: https://lnk.tu-bs.de/Ym3RKP
https://github.com/amkozlov/raxml-ng

Neighbor Joining (NJ) tree

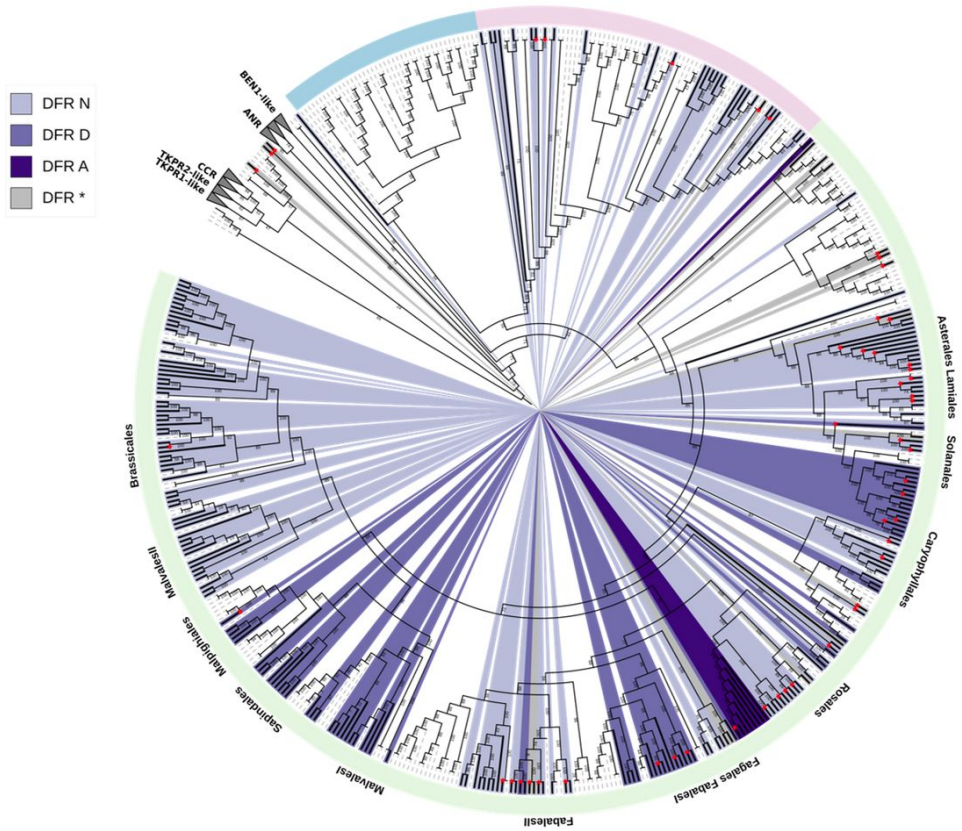
- Combination of sequences (groups of sequences) with smallest distance
- Quick and computationally cheap approach
- Less reliable than maximum likelihood (ML) trees
- Tool: MEGA



Kumar et al., 2016: 10.1093/molbev/msw054

EXAMPLE: gene tree of *DFR*

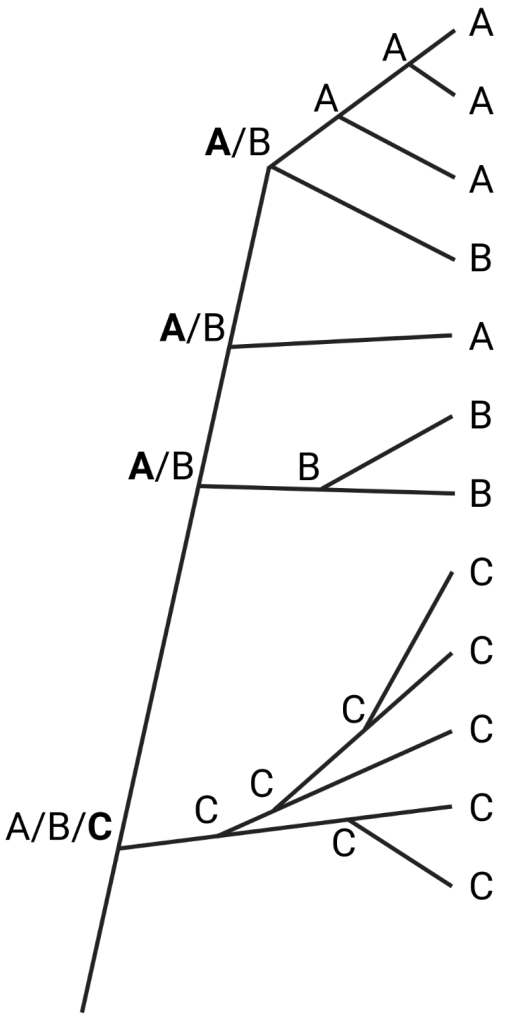
- Evolution of *DFR* in land plants
- Multiple clades visible
- Sequences of closely related species cluster in lineages



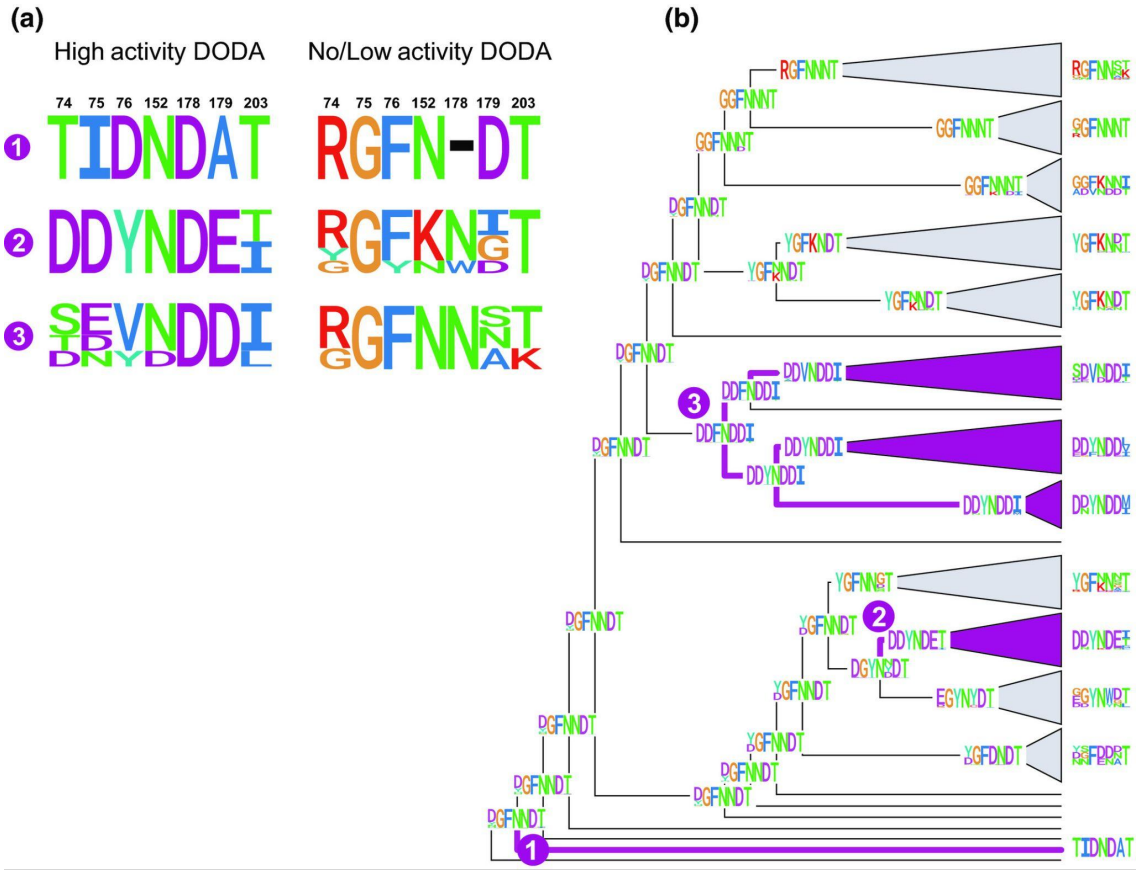
Choudhary & Pucker, 2023

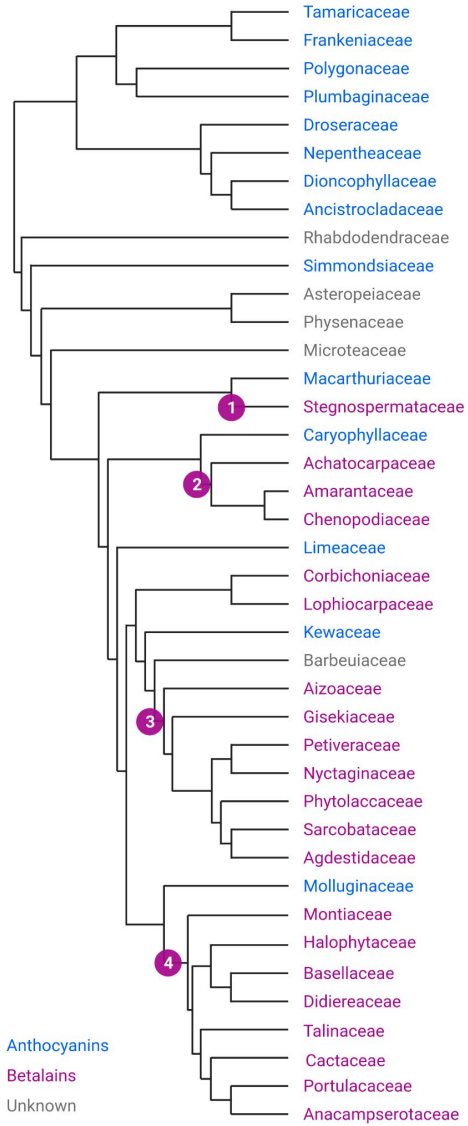
Ancestral state reconstruction

- Identify the state of a certain sequence/trait in a last common ancestor
- Multiple states are possible in some cases
- Ancestral states are often predicted with a probability



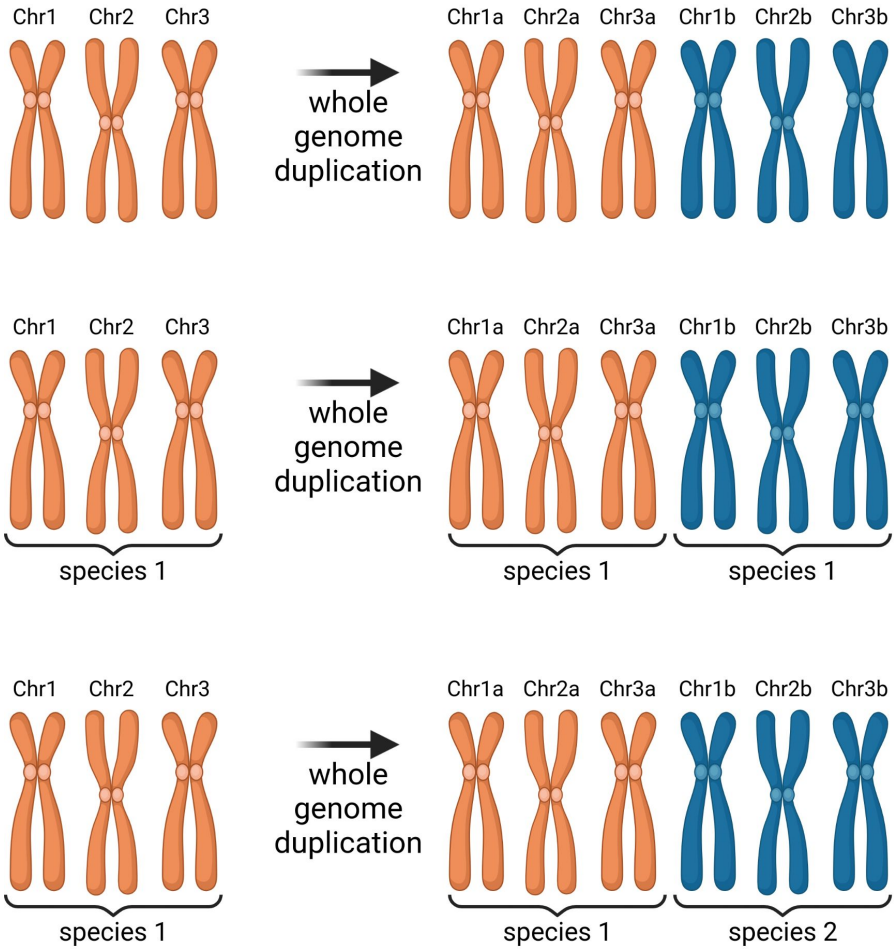
Example: Ancestral state reconstruction





Sheehan et al., 2020: 10.1111/nph.16089

Whole genome duplications



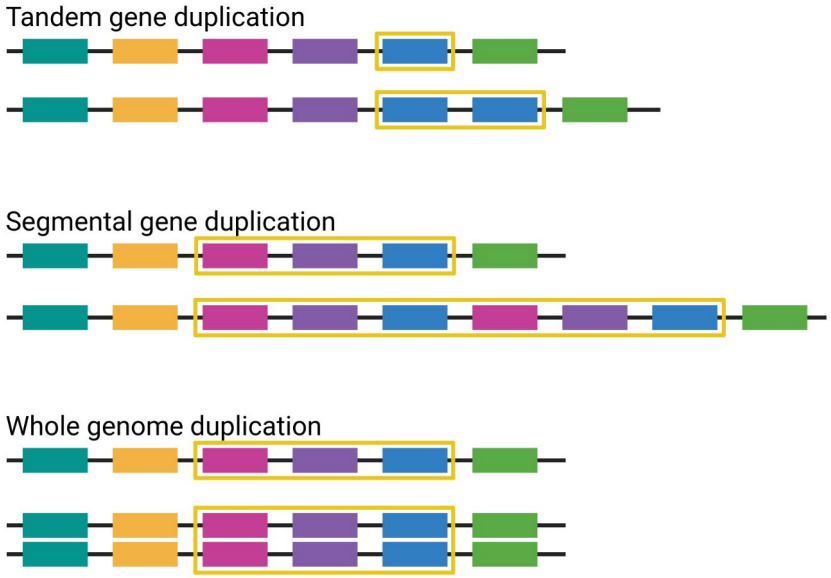
autopolyploidy

allopolyploidy

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4145113/

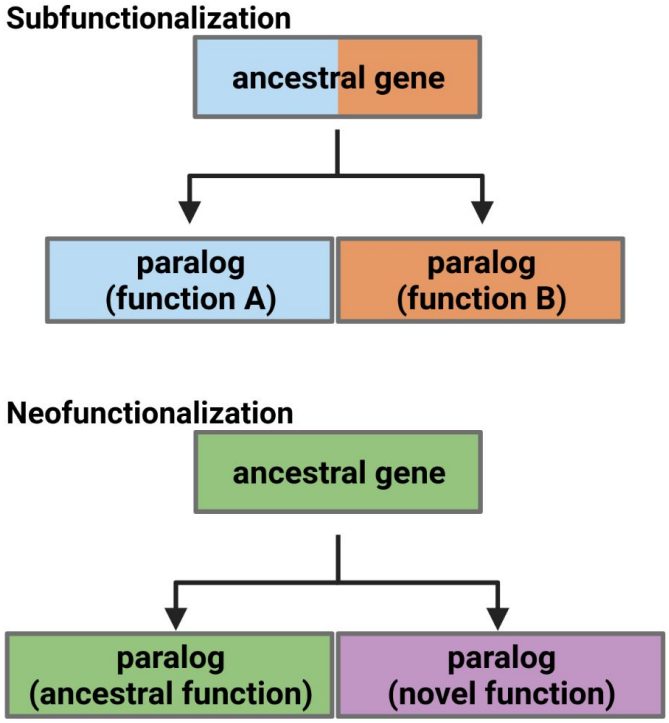
Classification of duplications: tandem, segment, WGD

- Gene copies can be the results of different mechanisms:
  - Tandem duplication: copies are located next to each other on the same chromosome
  - Segment duplication: like tandem duplications, but multiple adjacent genes are also duplicated
  - Whole genome duplication (WGD): all genes are duplicated

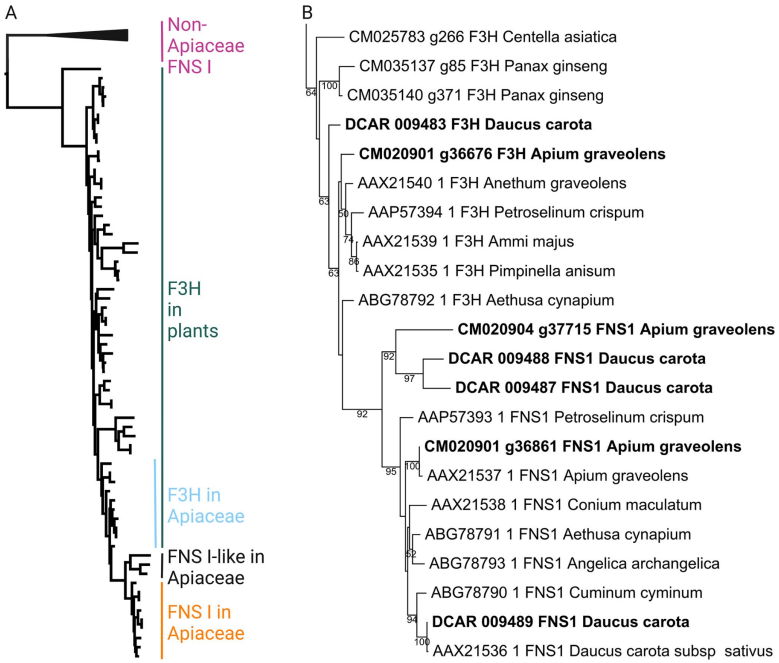


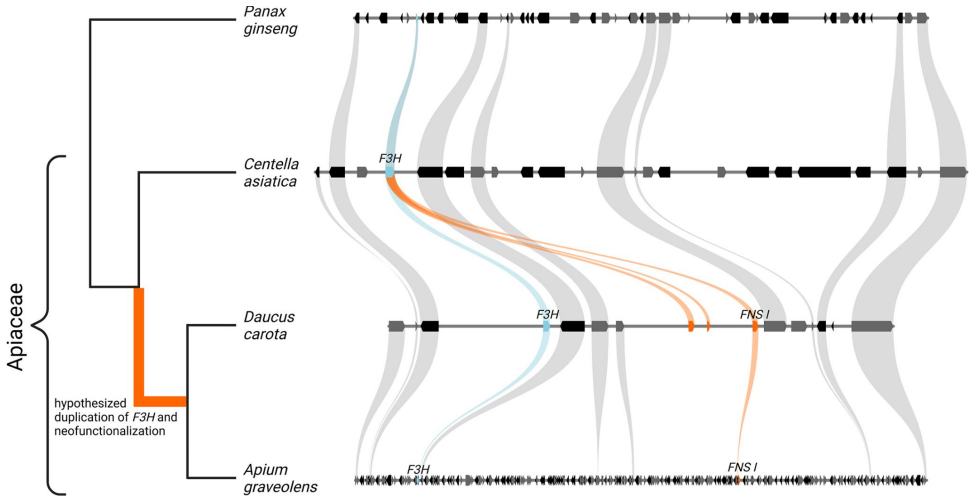
Subfunctionalization & Neofunctionalization

- Subfunctionalization: gene copies fulfill parts of the ancestral function
- Neofunctionalization: one copy maintains ancestral function, while other copy can acquire a novel function



EXAMPLE: *F3H*/*FNS I* neofunctionalization following gene duplication in Apiaceae





https://doi.org/10.1371/journal.pone.0280155

Summary

- Concepts of evolution
- Homology (orthology & paralogy)
- Alignment and phylogenetic tree construction
- Ancestral state reconstruction
- Neo- and subfunctionalization

Time for questions!

Questions

1. What is the difference between homolog, paralog, and ortholog?
2. What are the advantages/disadvantages of running BLAST locally vs. at the NCBI website?
3. What are suitable BLAST alternatives?
4. What is the difference between a local and a global alignment?
5. What are the different methods for the construction of a phylogenetic tree?
6. What is ancestral state reconstruction?
7. What are possible mechanisms underlying gene duplications?
8. What is neofunctionalization/subfunctionalization?
9. What is the relationship of A and B? (paralog/ortholog)
10. What is the relationship of D and E (paralog/ortholog)