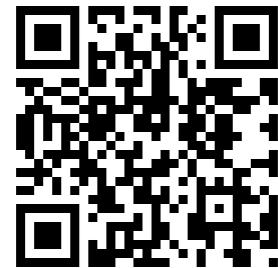


Prof. Dr. Boas Pucker

# **Phylogenetic Analyses**

# Availability of slides

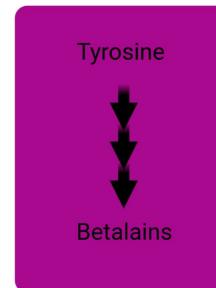
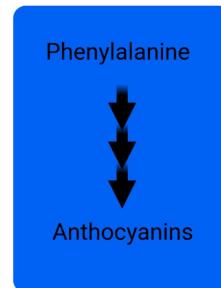
- All materials are freely available (CC BY) - after the lectures:
  - eCampus: WBIO-A-08
  - GitHub: <https://github.com/bpucker/teaching>
- Questions: Feel free to ask at any time
- Feedback, comments, or questions: [pucker\[a\]uni-bonn.de](mailto:pucker[a]uni-bonn.de)



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# Theodosius Dobzhansky (1900-1975)

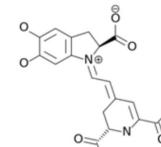
“Nothing in biology makes sense except in the light of evolution”



*Antirrhinum majus*



Anthocyanin color range

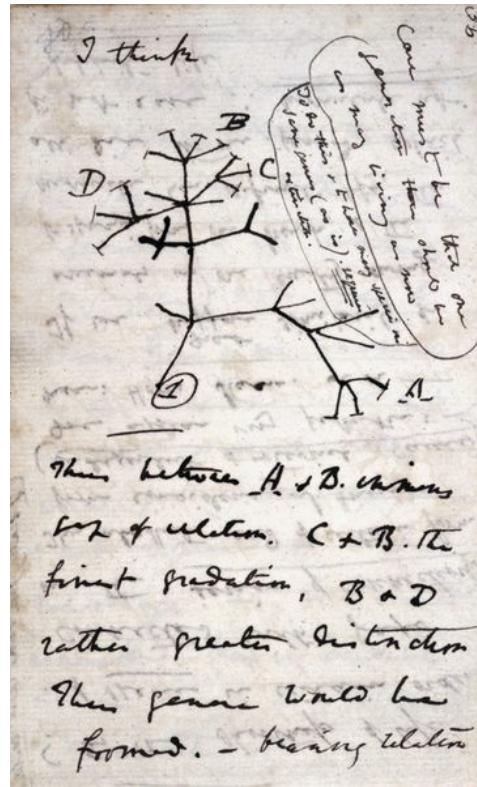
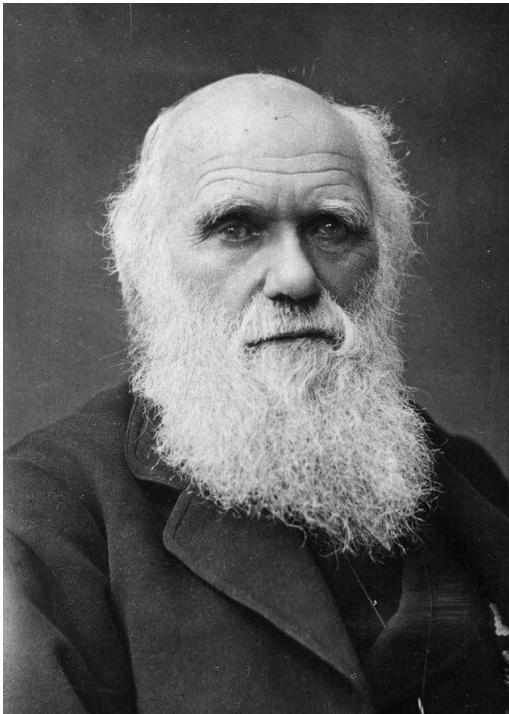


*Mirabilis jalapa*



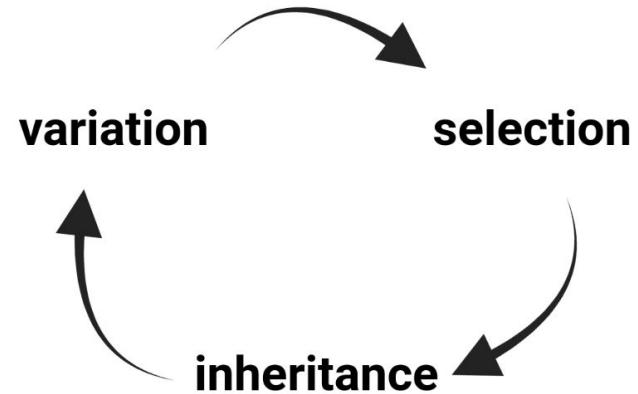
Betalain color range

# Charles Darwin (1809-1882)

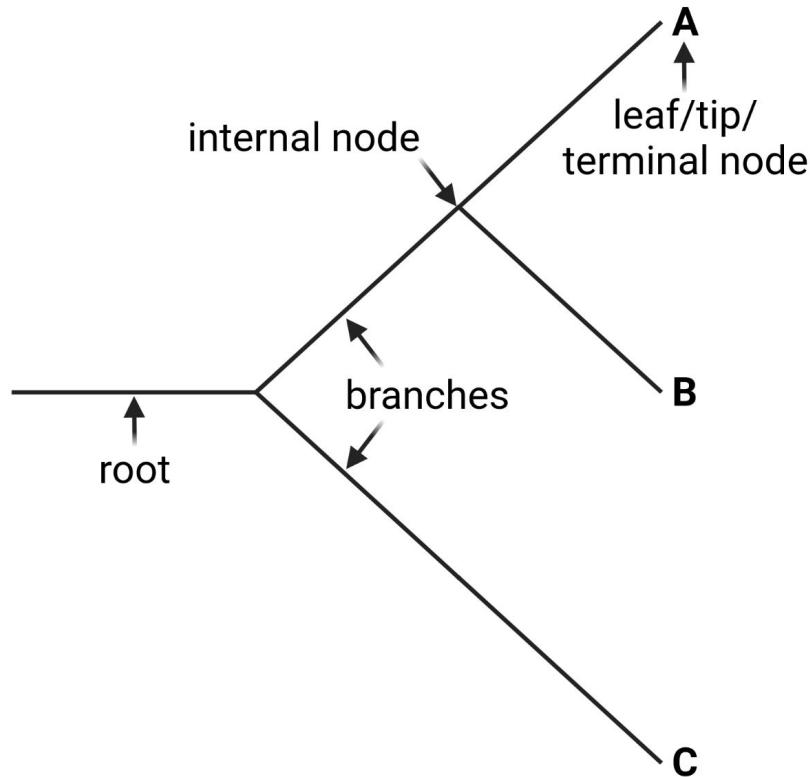


# Basic evolution concept

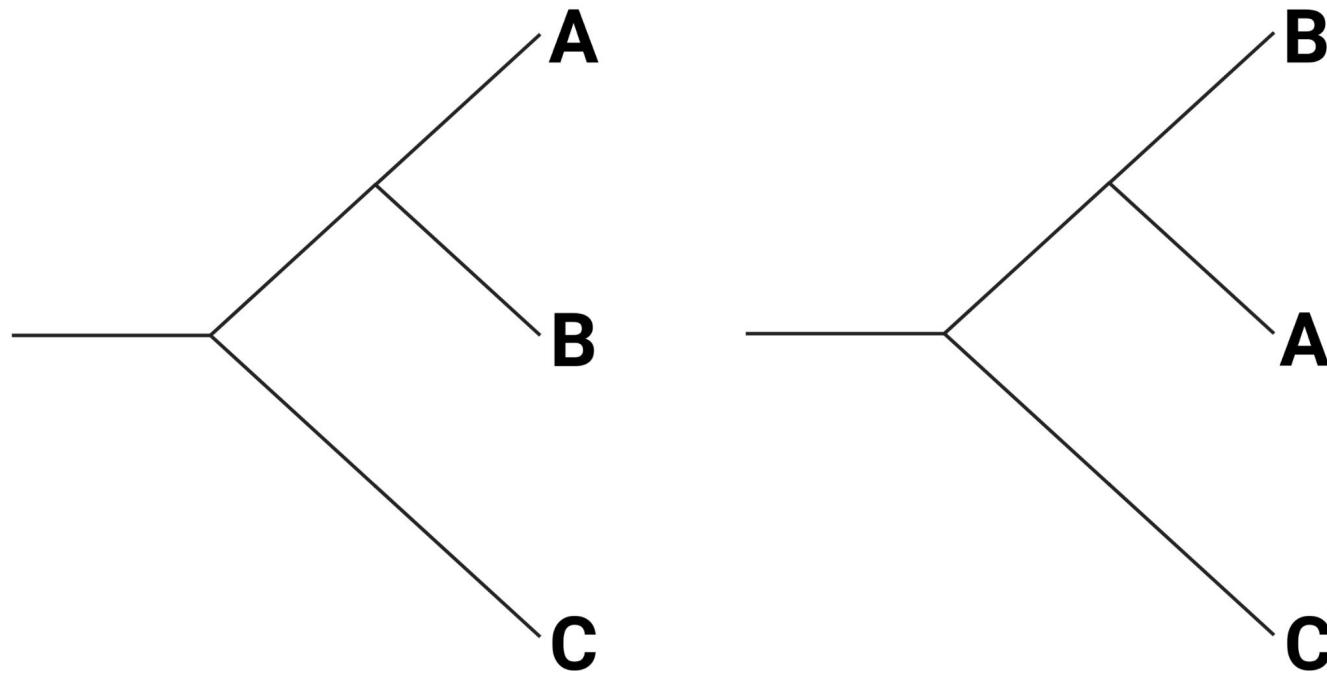
- Selection is performed by nature ('theory of natural selection')
- Selection requires (1) variation to act upon and (2) more offspring than sustainable
- Inheritance determines the properties of individuals (phenotype)



# Elements of a phylogenetic tree

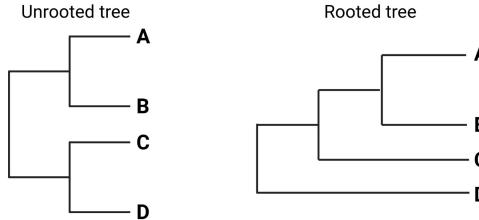


# Tree topology and rotation

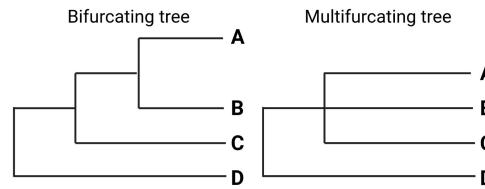


# Types of trees

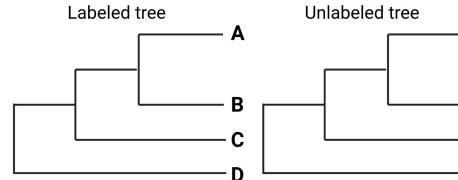
- Rooted vs. unrooted tree



- Bifurcating vs. multifurcating tree

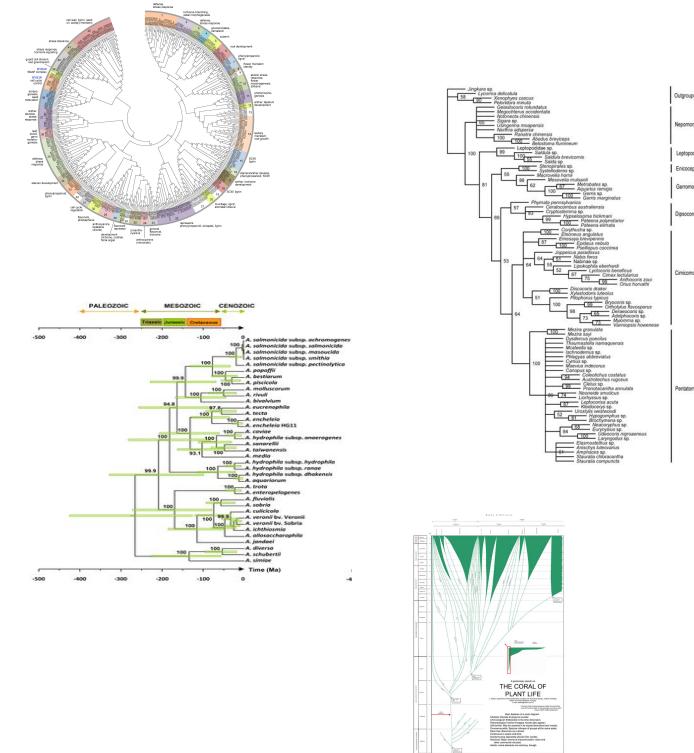


- Labeled vs. unlabeled trees



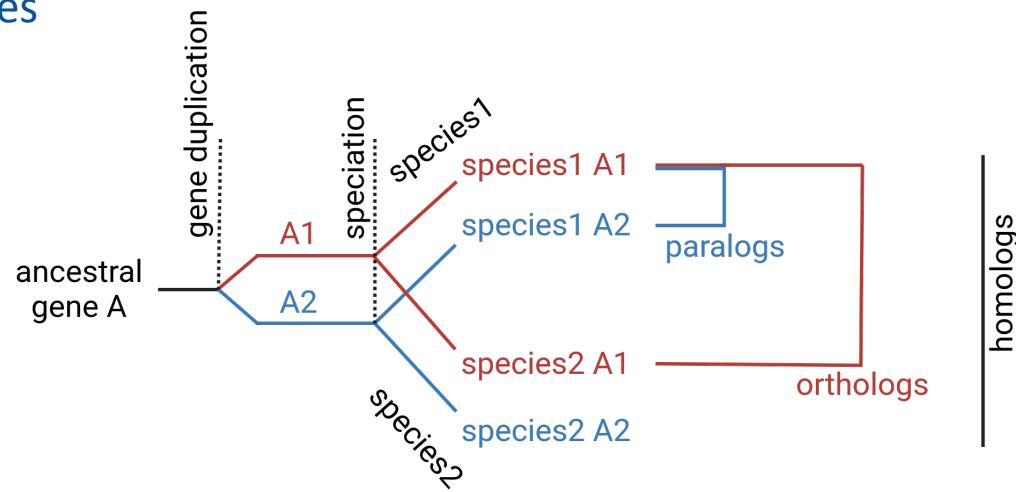
# Types of special trees

- 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



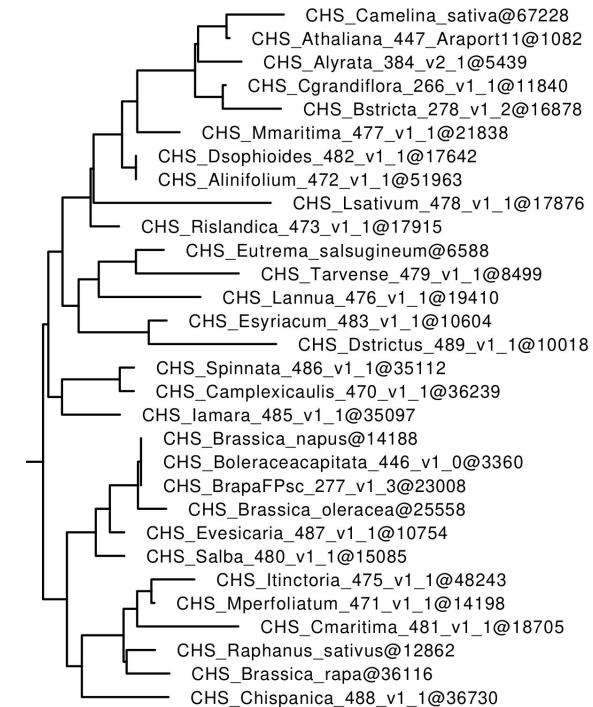
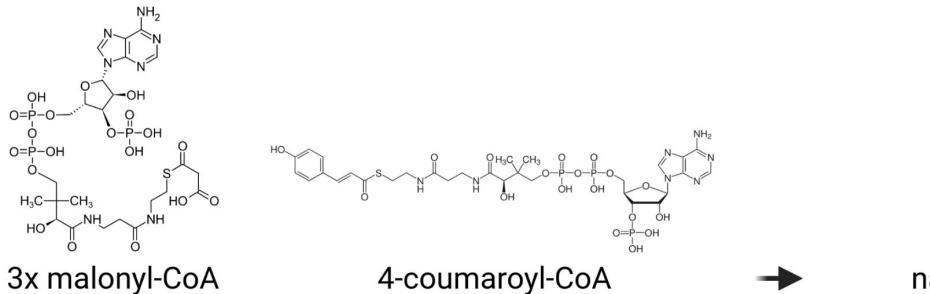
# Homologs, orthologs, and paralogs

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



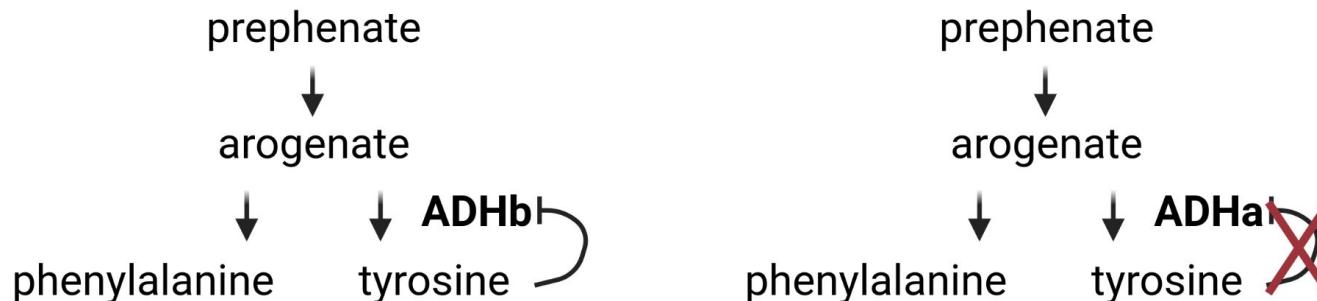
## Example: orthologs

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



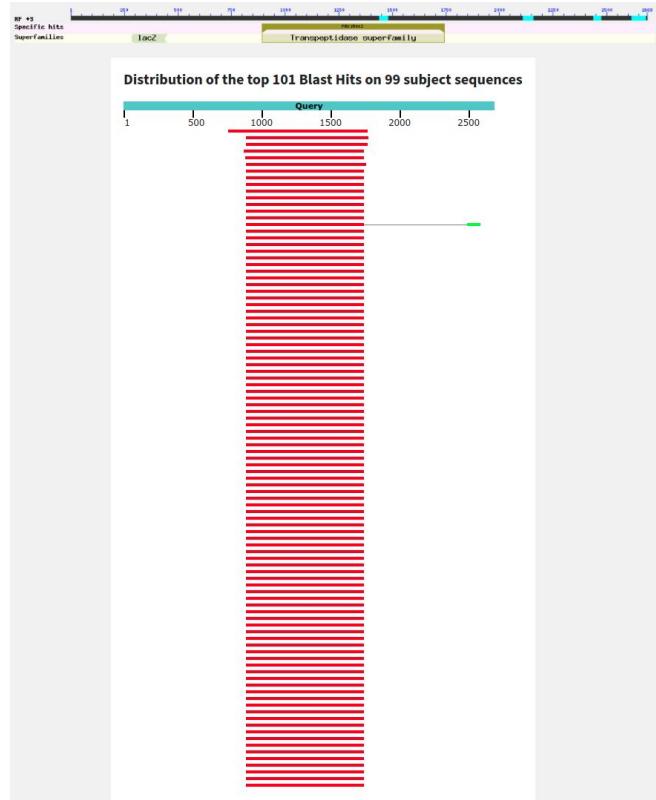
## Example: paralogs

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

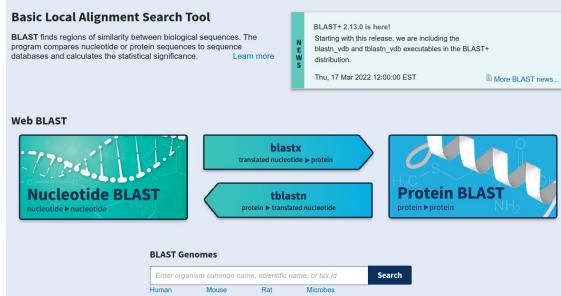


# BLAST - simple sequence searches

- 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



**Basic Local Alignment Search Tool**  
BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.

**NEWS**  
BLAST+ 2.13.0 is here!  
Starting with this release, we are including the blastn\_vdb and blastx\_vdb executables in the BLAST+ distribution.

Thu, 17 Mar 2022 12:00:00 EST      [More BLAST news...](#)

**Web BLAST**

- Nucleotide BLAST**: nucleotide ➔ nucleotide
- blastx**: translated nucleotide ➔ protein
- tblastn**: protein ➔ translated nucleotide
- Protein BLAST**: protein ➔ protein

**BLAST Genomes**  
Enter organism common name, scientific name, or tax id  
Human   Mouse   Rat   Microbes   [Search](#)

```

USAGE
blastn [-h] [-help] [-import_search_strategy filename]
[-export_search_strategy filename] [-task task_name] [-db database_name]
[-dblist dblist] [-taxid taxid] [-taxids taxids] [-taxidlist filename]
[-negative_taxid list filename] [-negative_taxids taxids] [-taxidlist filename]
[-negative_dblist dblist] [-enterz_query entrez_query]
[-tblastn] [-tblastn_vdb] [-tblastx] [-tblastx_vdb] [-tblastn_algorithm]
[-subject subject_input_file] [-subject_loc range] [-query query_input_file]
[-out output_file] [-evalval evalval] [-word_size int_value]
[-gapopen float_value] [-gapext float_value] [-gapext_perl float_value]
[-perc_identity float_value] [-scorw hsp_perc float_value]
[-max_hsps int_value] [-xdrop ungap float_value] [-xdrop_gap float_value]
[-xdrop_pgap float_value] [-xdrop_scoring float_value] [-penalty int_value]
[-reward int_value] [-no_greedy] [-min_raw_hsp_scoring float_value]
[-template_type type] [-template_length int_value] [-dust DUST_options]
[-filtering filter_type] [-log_database]
[-window_masker window_masker_db] [-soft_softmasking soft_softmasking]
[-window_masker_db window_masker_db] [-soft_softmasking soft_softmasking]
[-ungapped] [-culling_limit int_value] [-best_hit_overhang float_value]
[-best_hit_percent_id float_value] [-max_hsps int_value]
[-window_size int_value] [-off_diagonal range int_value]
[-use_index boolean] [-index_name string] [-lcase_lmasking]
[-query query] [-target target] [-strand strand] [-pad pad] [-decoy decoy] [-output_format]
[-labeled] [-labeled_description labeled_val] [-labeled_alignments int_value]
[-line_length line_length] [-html] [-sorthits sort_hits]
[-sorths sort_https] [-max_target_seqs num_sequences]
[-num_threads int_value] [-remote] [-version]

DESCRIPTION
  Nucleotide-Nucleotide BLAST 2.9.0+

```

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

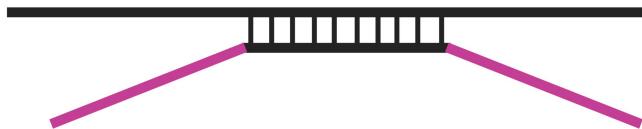
# BLAST alternatives

- HMMER: <http://hmmer.org/>
  - Dedicated to the identification of specific domains
- DIAMOND: <https://github.com/bbuchfink/diamond>
  - Much faster, but higher memory requirements

# 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

Local alignment



Global alignment



# MAFFT: online vs. local

## Multiple Sequence Alignment

MAFFT (Multiple Alignment using Fast Fourier Transform) is a high speed multiple sequence alignment program.

We have recently changed the default parameter settings for MAFFT. Alignments should run much more quickly and larger DNA alignments can be carried out by default. Please click the 'More options' button to review the defaults and change them if required.

**Important note:** This tool can align up to 500 sequences or a maximum file size of 1 MB.

STEP 1 - Enter your input sequences

Enter or paste a set of  
AUTOMATIC

sequences in any supported format:

Or upload a file:  No file selected.

Use a example sequence | Clear sequence | See more example inputs

STEP 2 - Set your Parameters

OUTPUT FORMAT  
Pearson/Fasta

The default settings will fulfill the needs of most users.  
 (Click here, if you want to view or change the default settings.)

STEP 3 - Submit your job

Be notified by email (Tick this box if you want to be notified by email when the results are available)

```
-----  
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)
```

# 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  
MDTDIKYSEKWIQGHSIFPDQQ  
>seq3  
METEKYMEKWIQGHSIFPEDQ  
>seq4  
MDTEIKYMEKWIQGHALFPDDQP
```

Alignment of sequences



seq1	MDTE-KYMEKWIDQGHALFPEEDQ-
seq2	MDTD-IKYSEKWI-QGHSIFPD-DQ-
seq3	METE-KYMEKWI-QGHSIFPE-DQ-
seq4	MDTE-IKYMEKWI-QGHALFPD-DQP

# Alignment formats

## CLUSTAL

AthCHS	MVMAGASSLDEIRQAQRADGPAGILAIGTANPENHVLOAEYPDYYFRITNSEHMTDLKEK
BvuCHS	M---ATPSVQEIRDAQRNSNGPATILAIGTANPANEMYQAEYPDFYFRVTKSEHMSSELKQK
	* .:.*:***:***:*** **** * .: * ***:***:***:***:***:*
AthCHS	FKRMCDKSTIRKRHMHLTEFLKENPHMCAYMAPSLDTRQDIVVVEVPKLGKEAAVKAIAK
BvuCHS	FKRMCDKSMIKKRYMHVTQELLEENPHMCDYNASSLNTRQDILATEVPKLGKEAAVKAIAK
	***** *:***:***:***:***:***** * .:***:*****:..*****:*****:*****
AthCHS	EWGQPDKSITHVVFCTTSVGDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRIAK
BvuCHS	EWGQPRSKITHVIFCTTSVGDMPGADYQLTKLLGLRPSVKRFMLYQQCYAGGTVLRLAK
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****
AthCHS	DIAENNRRGARVLVVCEITAVTFRGSDTHLDLSVGQALFSDGAALIVGSDPDTSVGEEK
BvuCHS	DIAENNRRGARVLVVCAEITVICFRGPTETHLDMSGQALFGDGAGAVIVGADLDESI-ER
	*:*****:*****:***: .: * ***:*****:*****:***: .:***:***:***:*
AthCHS	PIFEMVSAAQTILPDSDGAIDGHLREVGLTFHLLKDVPGLISKNIVKSLDEAFKPLGISD
BvuCHS	PIFQLWAAQTILPDSEGAIDGHLREVGLAFHLLKDVPGLISKNIEKALVEAFKPIGIDD
	***:***:*****:*****:*****:*****:*****:***:***:***:***:*
AthCHS	WNSLFWIAHPGGPAILDQVEIKLGLKEEKMRATRHLSEYGNMSSACVLFILDEMRRKSA
BvuCHS	WNSIFWAAPGGPAILDQVESKGLKQDKLSTTRHVLSEFGNMSSACVLFILDEMRRKRSM
	***:***:*****:*****:***: .:*****:*****:*****:***:*
AthCHS	KDGVATTGEGLEWGVLFGFGPGLTVETVVLHSVPL--
BvuCHS	KEGMATTGEGLEWGVLFGFGPGLTVETVVLHSVPIAN
	*:*****:*****:*****:*****:*****:

## FASTA

>AthCHS

MVMAGASSLDEIRQAQRADGPAGILAIGTANPENHVLOAEYPDYYFRITNSEHMTDLKEK  
 FKRMCDKSTIRKRHMHLTEFLKENPHMCAYMAPSLDTRQDIVVVEVPKLGKEAAVKAIAK  
 EWGQPDKSITHVVFCTTSVGDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRIAK  
 DIAENNRRGARVLVVCEITAVTFRGSDTHLDLSVGQALFSDGAALIVGSDPDTSVGEK  
 PIFEMVSAAQTILPDSDGAIDGHLREVGLTFHLLKDVPGLISKNIVKSLDEAFKPLGISD  
 WNSLFWIAHPGGPAILDQVEIKLGLKEEKMRATRHLSEYGNMSSACVLFILDEMRRKSA  
 KDGVATTGEGLEWGVLFGFGPGLTVETVVLHSVPL--

>BvuCHS

M---ATPSVQEIRDAQRNSNGPATILAIGTANPANEMYQAEYPDFYFRVTKSEHMSSELKQK  
 FKRMCDKSMIKKRYMHVTQELLEENPHMCDYNASSLNTRQDILATEVPKLGKEAAVKAIAK  
 EWGQPRSKITHVIFCTTSVGDMPGADYQLTKLLGLRPSVKRFMLYQQCYAGGTVLRLAK  
 DIAENNRRGARVLVVCAEITVICFRGPTETHLDMSGQALFGDGAGAVIVGADLDESI-ER  
 PIFQLWAAQTILPDSEGAIDGHLREVGLAFHLLKDVPGLISKNIEKALVEAFKPIGIDD  
 WNSIFWAAPGGPAILDQVESKGLKQDKLSTTRHVLSEFGNMSSACVLFILDEMRRKSM  
 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-KYMEKWIDQGHALFPEDQ-
seq2	MDTDIKYSEKWI-QGHSLFPD-DQ-
seq3	METE-KYMEKWIDQHSIFPE-DQ-
seq4	MDTEIKYMEKWIDQGHALFPD-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

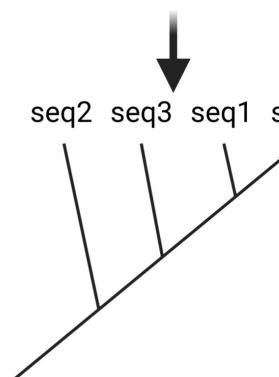
Trimmed alignment of sequences

seq1	MDTE - KYMEKWIQGHALFPEDQ
seq2	MDTD IKYSEKWIQGHSLFPDDQ
seq3	METE - KYMEKWIQGHSIFPEDQ
seq4	MDTEIKYMEKWIQGHALFPDDQ

Distance calculation

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

seq2 seq3 seq1 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)

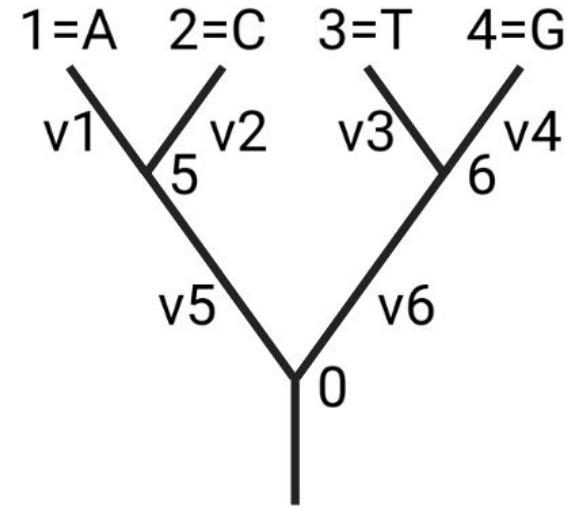
ATG GAT ACT GAA ...



seq 1	MDTE - KYMEKWIDQGHALFPEEDQ -
seq 2	MDTDIKYSEKWI - QGHSLFPD - DQ -
seq 3	METE - KYMEKWI - QGHSIFPE - DQ -
seq 4	MDTEIKYMEKWI - QGHALFPD - DQP

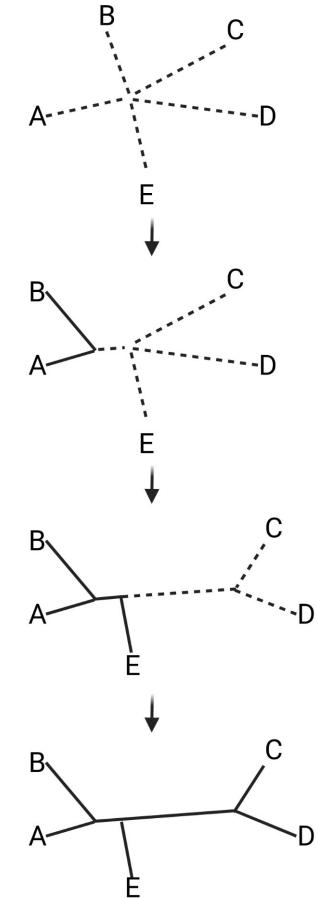
# Maximum Likelihood (ML) trees

- 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



# Neighbor Joining (NJ) tree

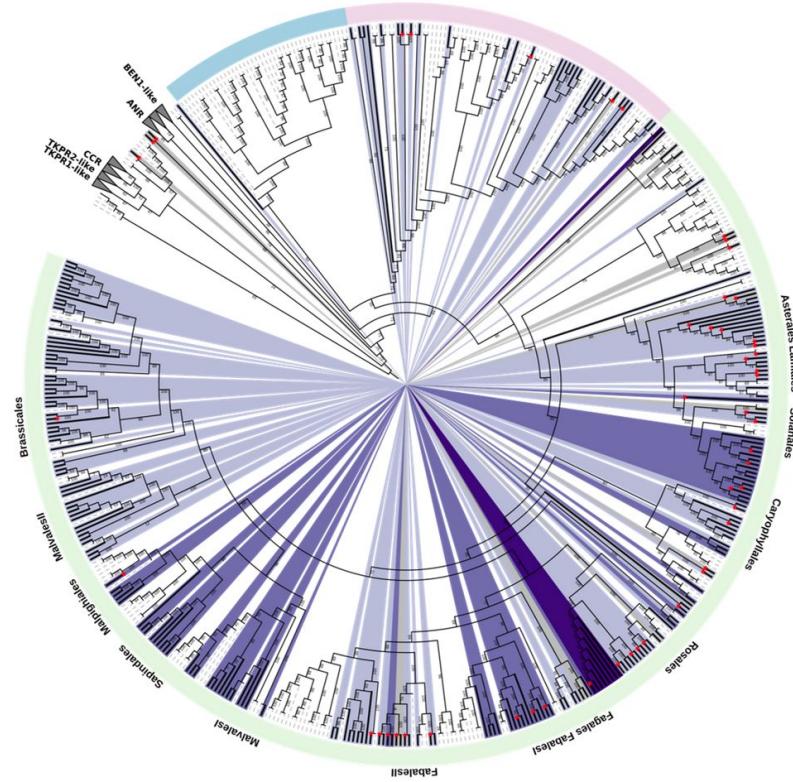
- Combination of sequences (groups of sequences) with smallest distance
- Quick and computationally cheap approach
- Less reliable than maximum likelihood (ML) trees
- Tool: MEGA



# EXAMPLE: gene tree of *DFR*

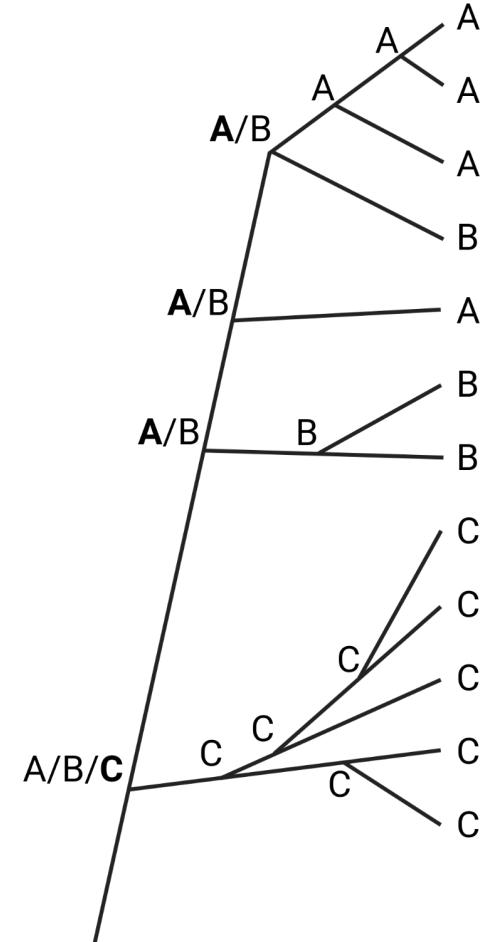
- Evolution of *DFR* in land plants
- Multiple clades visible
- Sequences of closely related species cluster in lineages

DFR N  
DFR D  
DFR A  
DFR \*



# 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

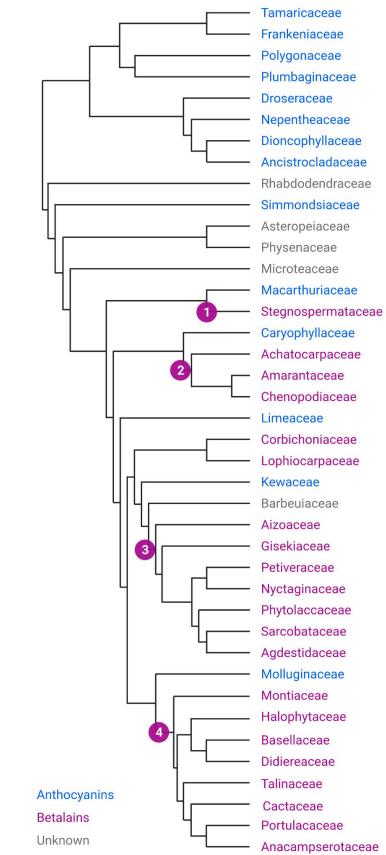
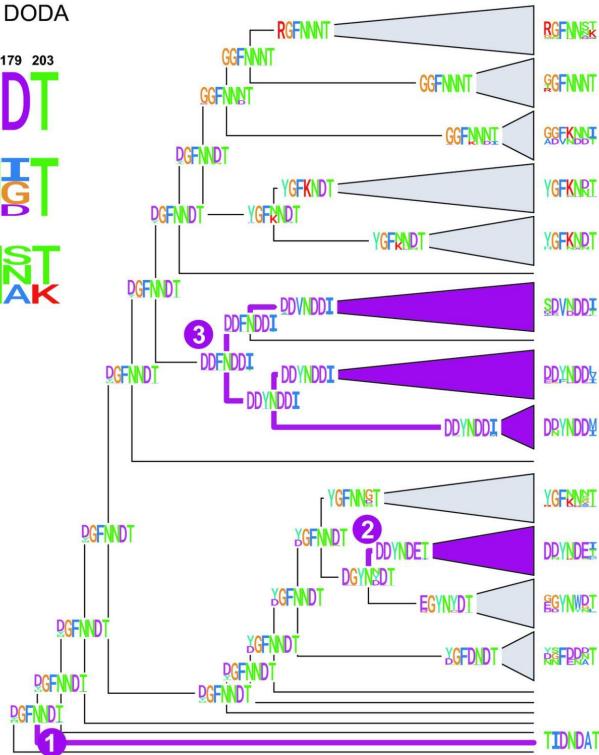


# Example: Ancestral state reconstruction

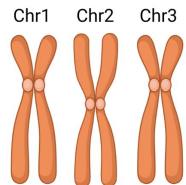
(a)

High activity DODA	No/Low activity DODA
74 75 76 152 178 179 203	74 75 76 152 178 179 203
① <b>TIDNDAT</b>	<b>RGFN-DT</b>
② <b>DDYNDEI</b>	<b>RGEKNGT</b>
③ <b>SEVNDDI</b>	<b>RGFNNSTAK</b>

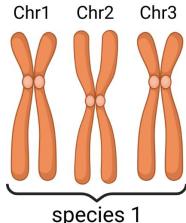
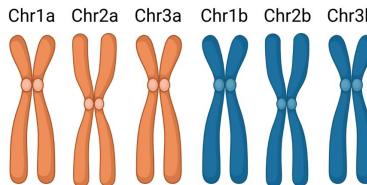
(b)



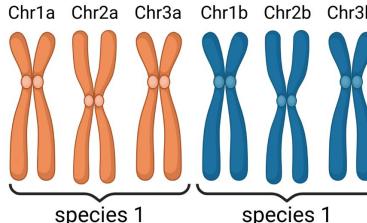
# Whole genome duplications



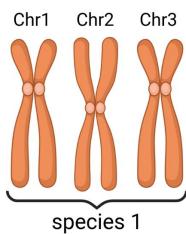
whole  
genome  
duplication



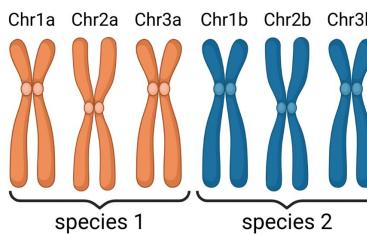
whole  
genome  
duplication



autopolyploidy



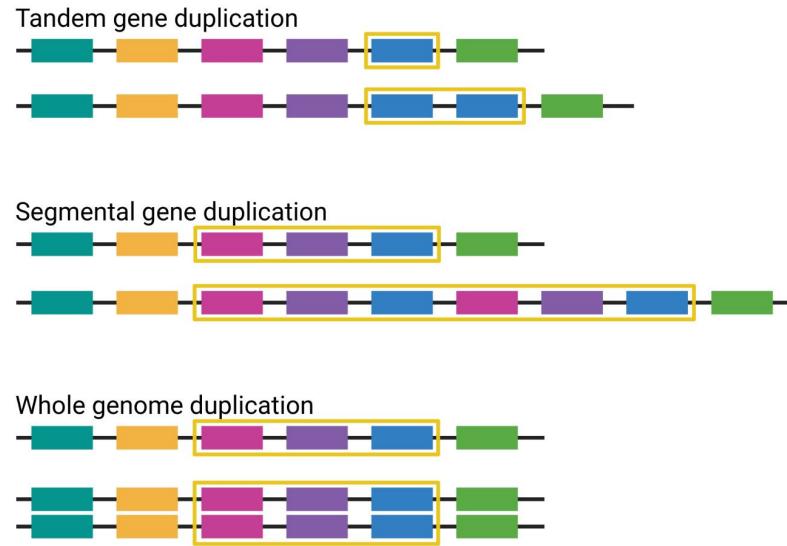
whole  
genome  
duplication



allopolyploidy

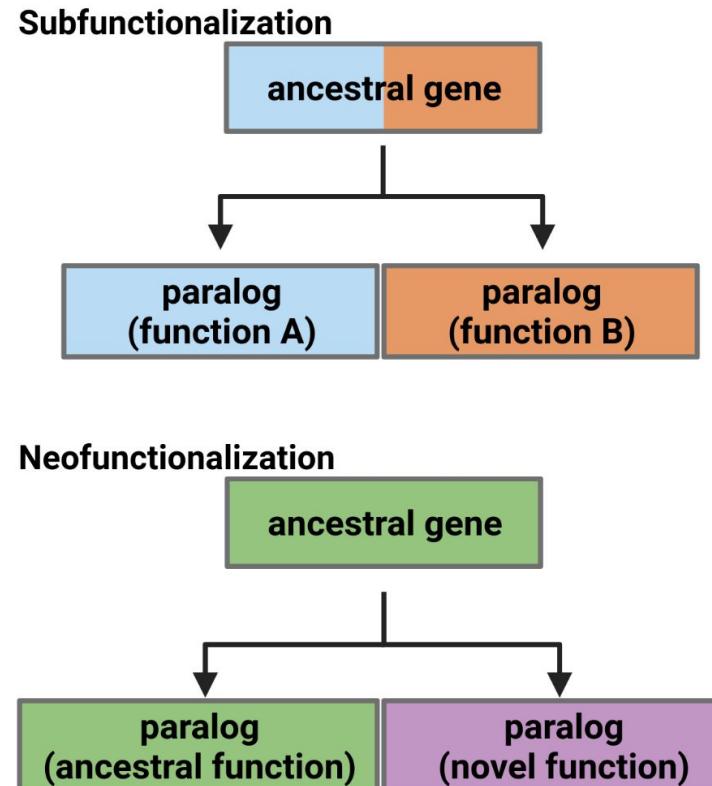
# Classification of duplications: tandem, segment, WGD

- 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

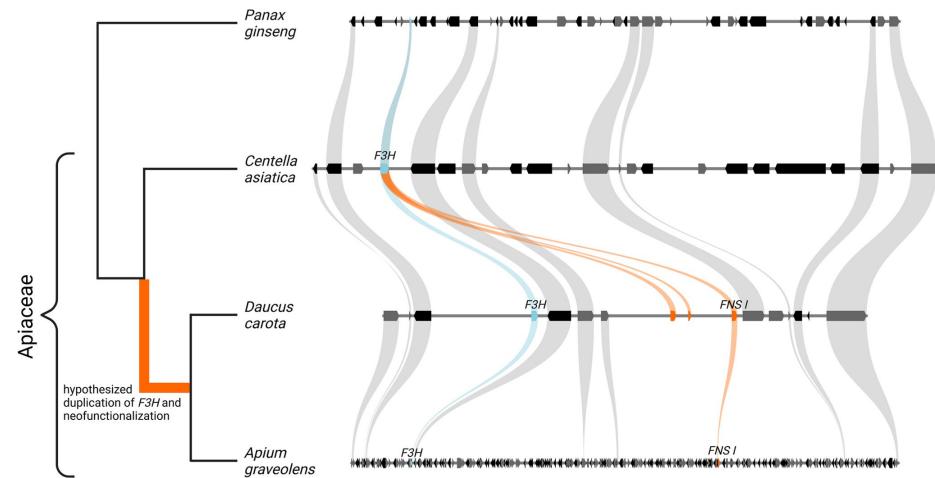
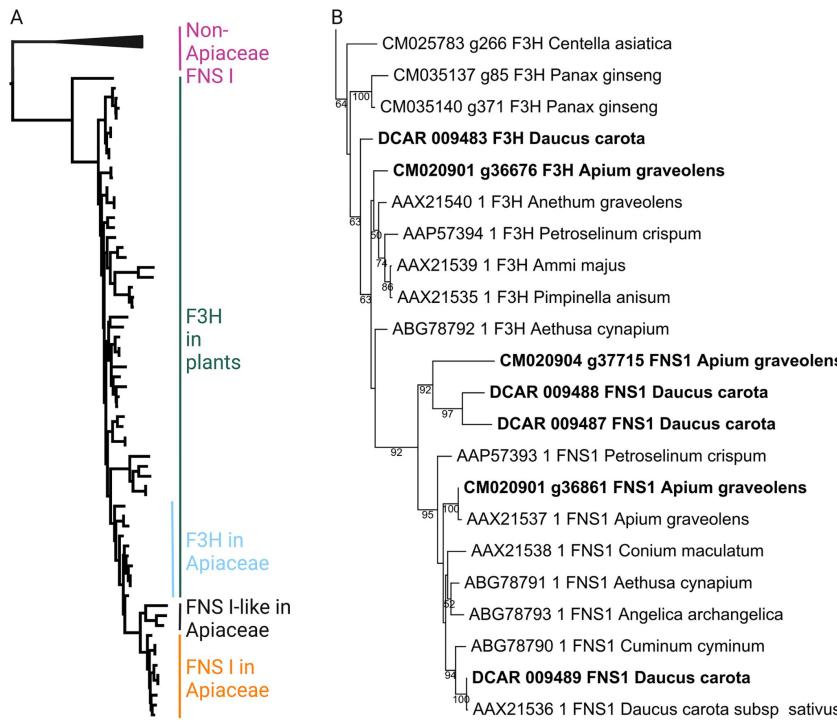


# Subfunctionalization & Neofunctionalization

- 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* / neofunctionalization



- 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)

