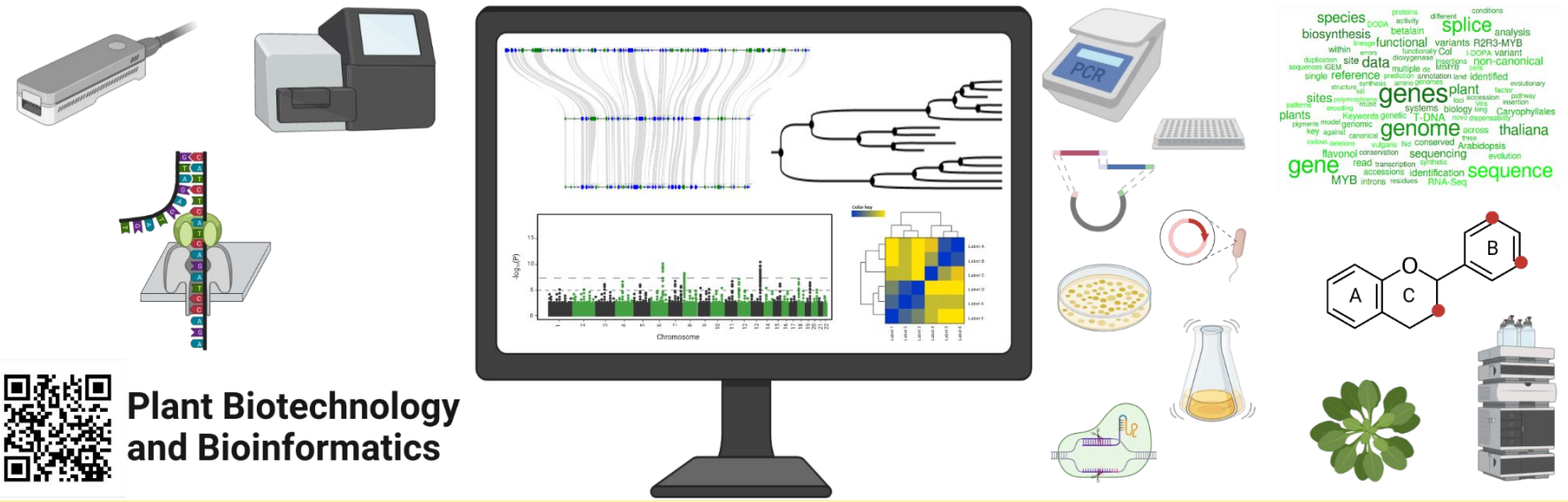
Technische Universität Braunschweig



Plant Biotechnology and Bioinformatics

Flavonoids

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

Availability of slides

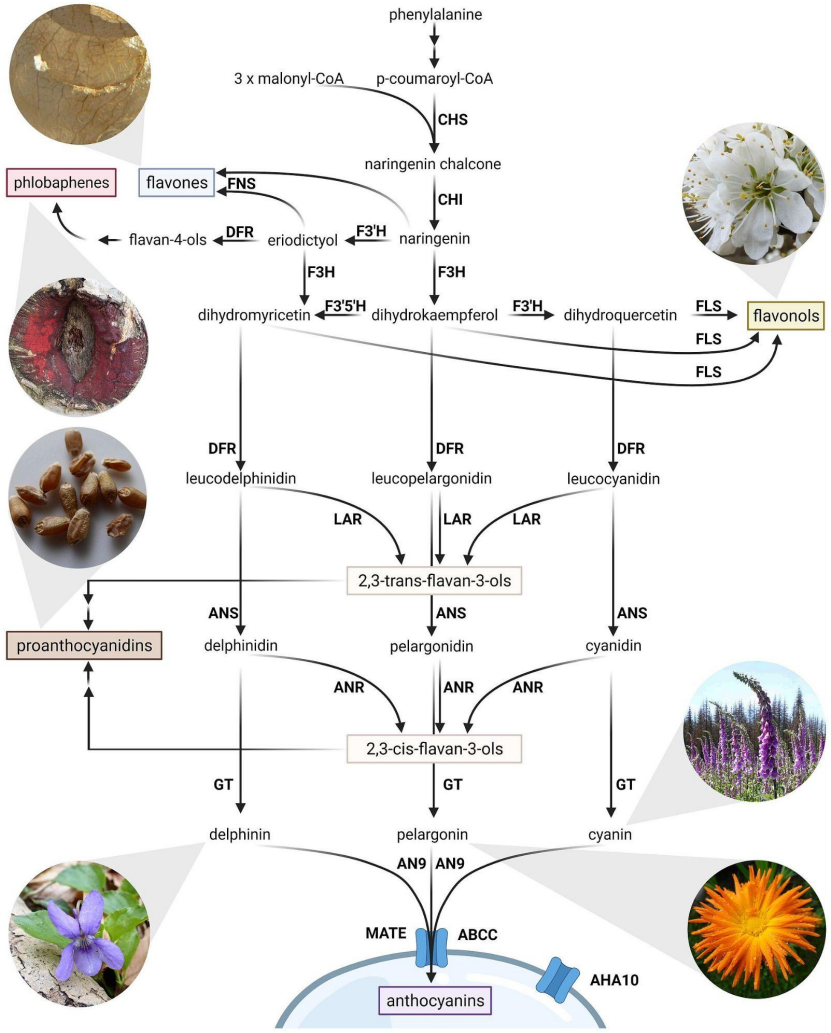
- All materials are freely available (CC BY) - after the lectures:
  - StudIP: LMChemBSc12
  - GitHub: https://github.com/bpucker/teaching

- Questions: Feel free to ask at any time

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

Anthocyanins: colorful compounds with health benefits

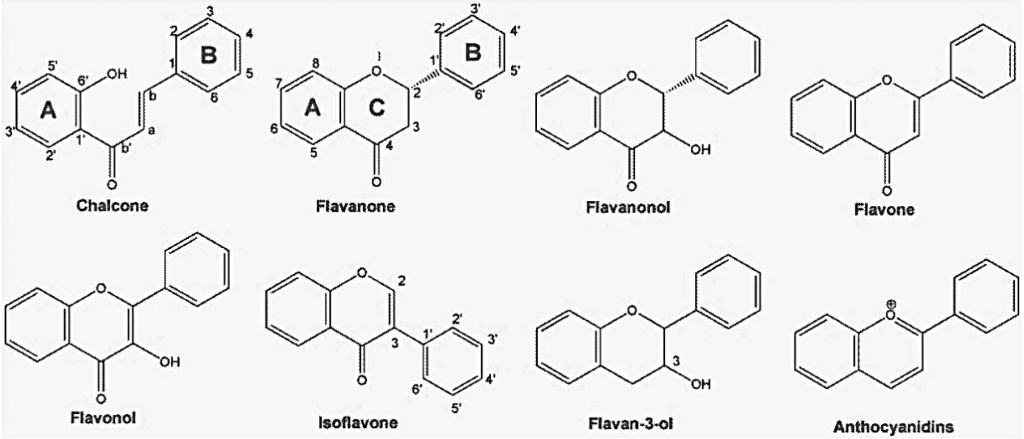
Branches of the flavonoid biosynthesis



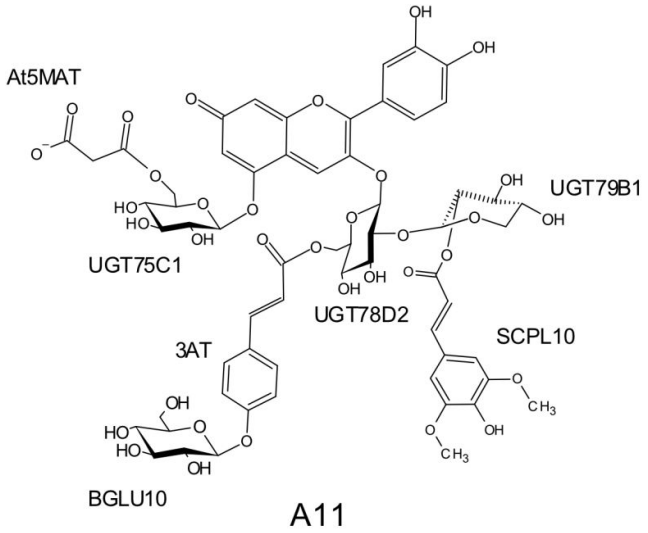
flavones: https://de.wikipedia.org/wiki/Heimkr%C3%A4uter#/media/Datei:Salazaria_mexicana_8.jpg

https://www.tu-braunschweig.de/en/ifp/pbb

Chemical diversity of flavonoids

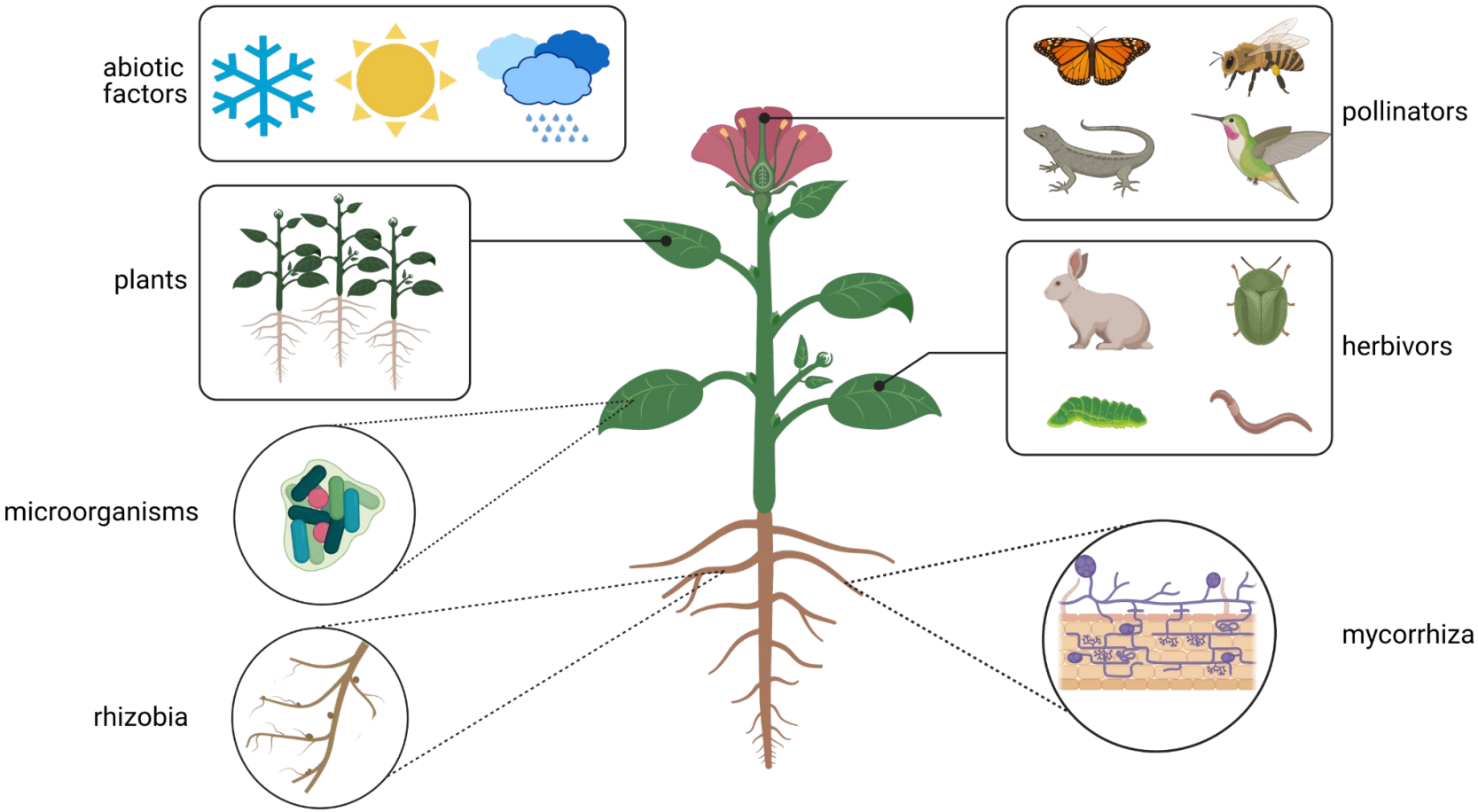


Over 8,000 different flavonoids reported so far



Bloor & Abrahams, 2002: 10.3390/molecules19033570

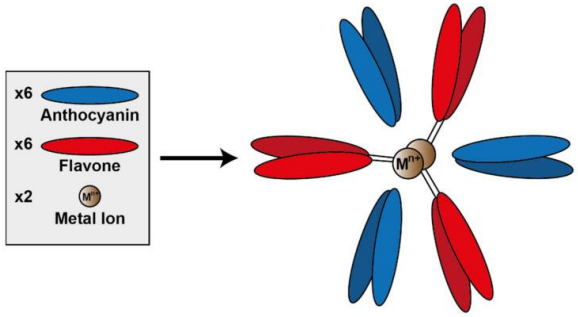
Why do plants produce many different flavonoids?



Flavones

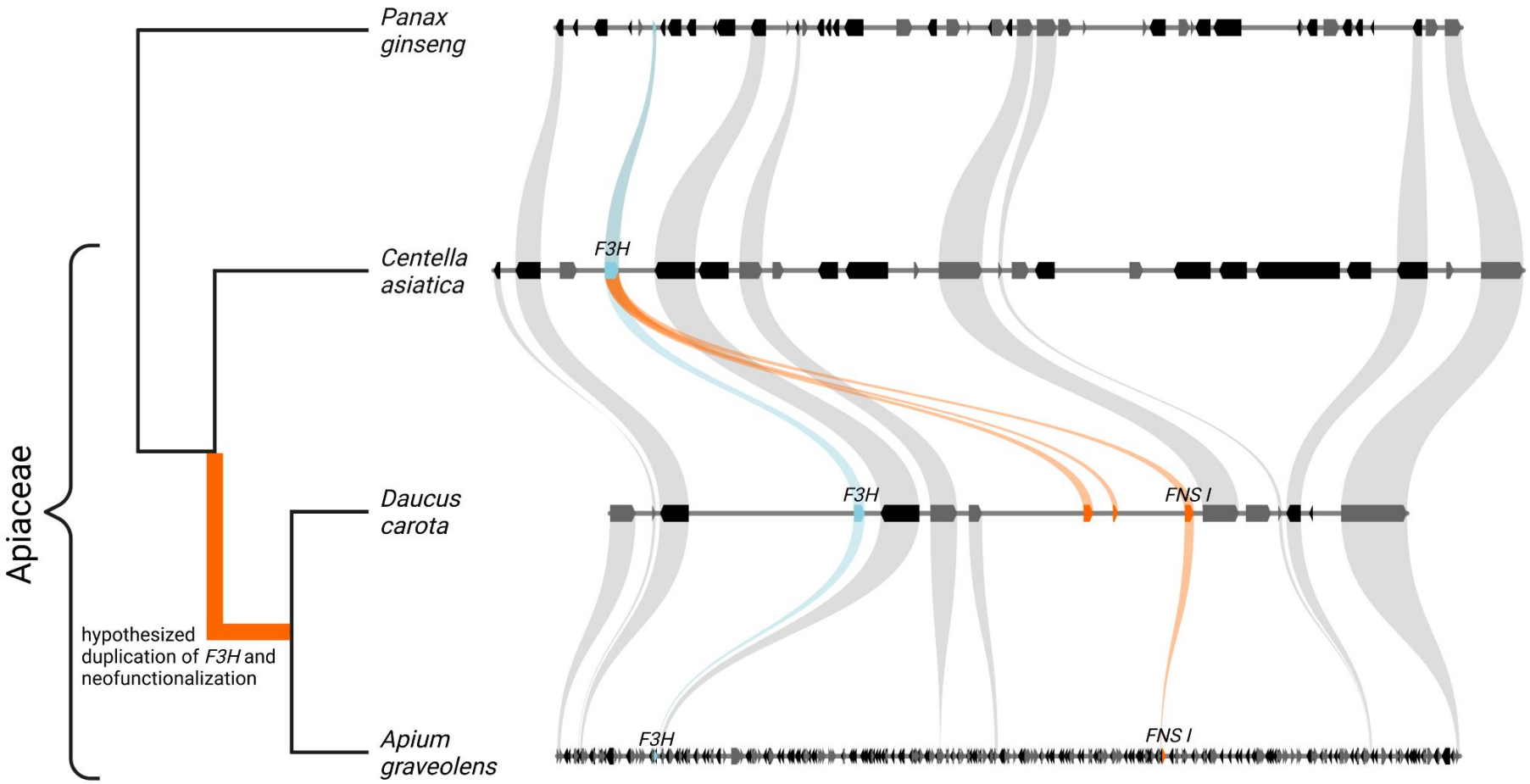
- Evolutionary old branch of flavonoid biosynthesis
- Antioxidant activity
- Associated with UV protection
- White or pale yellow pigmentation
- Co-pigmentation with other flavonoids
- Providing protection against insects, fungal infections
- Promoting colonization of roots by nitrogen-fixing bacteria and mycorrhizal fungi

wild carrot



Hostetler *et al.*, 2017: 10.3945/an.116.012948
Yu *et al.*, 2021: 10.1038/s41477-021-00897-y
Houghton *et al.*, 2021: 10.3390/plants10040726

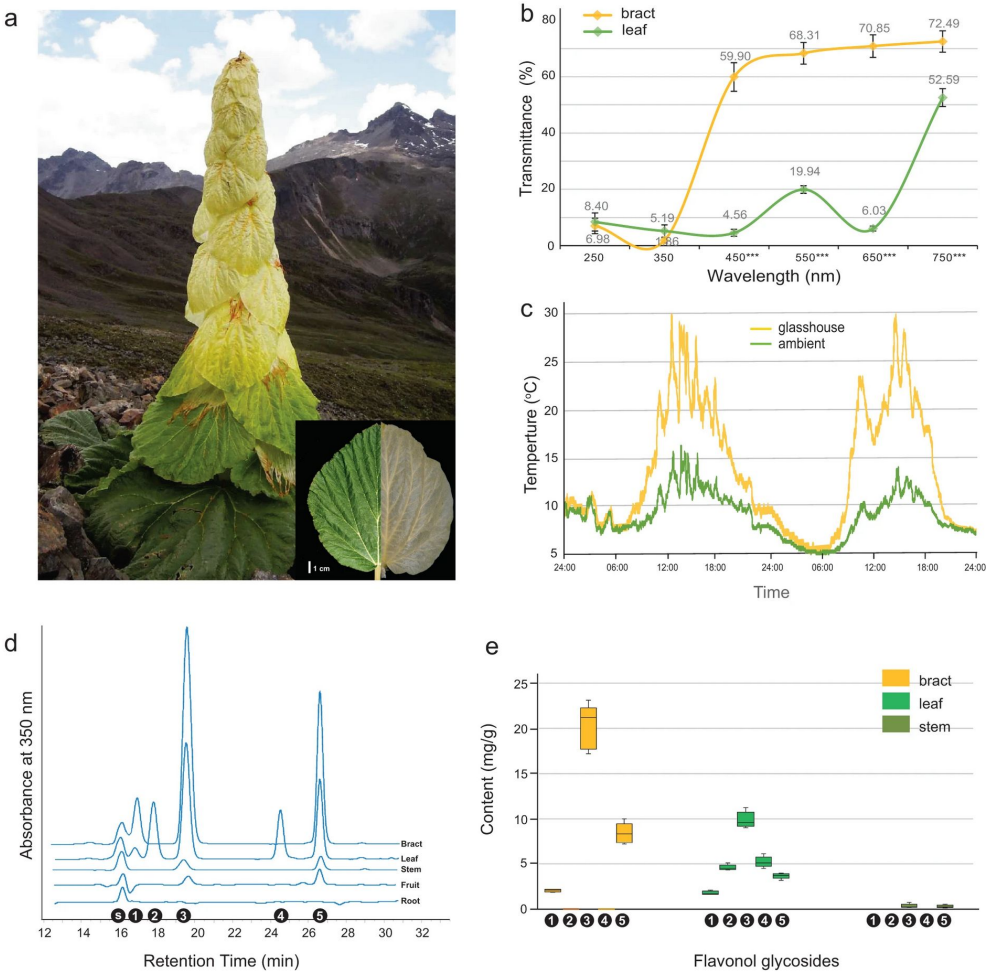
Example: Evolution of the flavone biosynthesis in Apiaceae



Pucker & Iorizzo, 2023: 10.1371/journal.pone.0280155

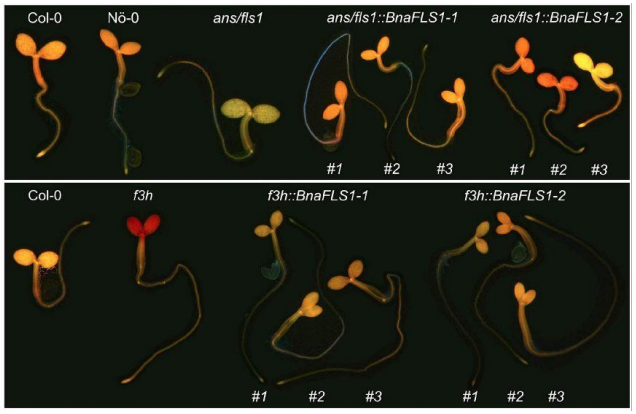
Flavonols

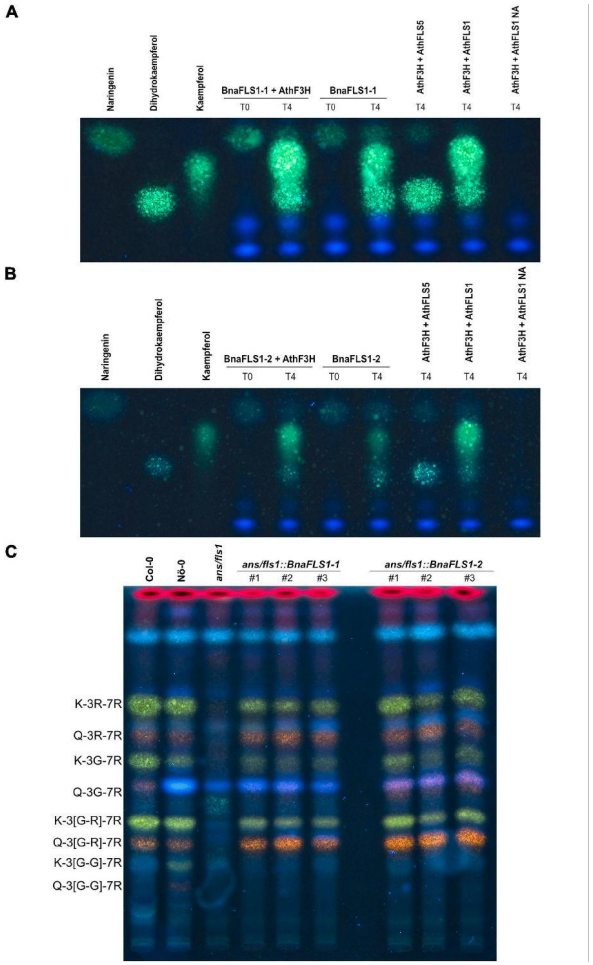
- Associated with UV protection
- Antioxidant activity
- White or pale yellow pigmentation
- Co-pigmentation with other flavonoids



https://doi.org/10.1038/s42003-023-05044-1

Flavonol analysis via staining

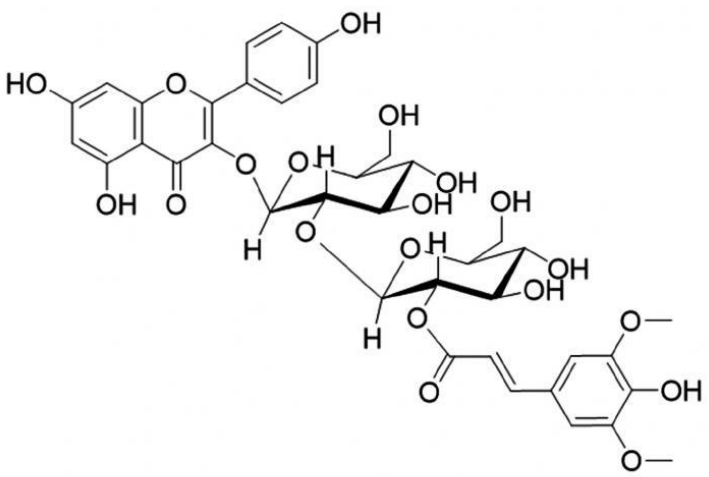




Schilbert et al., 2021: 10.3389/fpls.2021.733762

Example: bitter tasting kaempferols in rapeseed

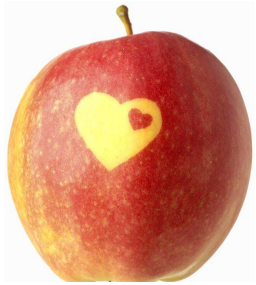
- Kaempferol 3-O-(2'''-O-sinapoyl-β-sophoroside)
- K3OSS prevents use of rapeseed protein for human nutrition



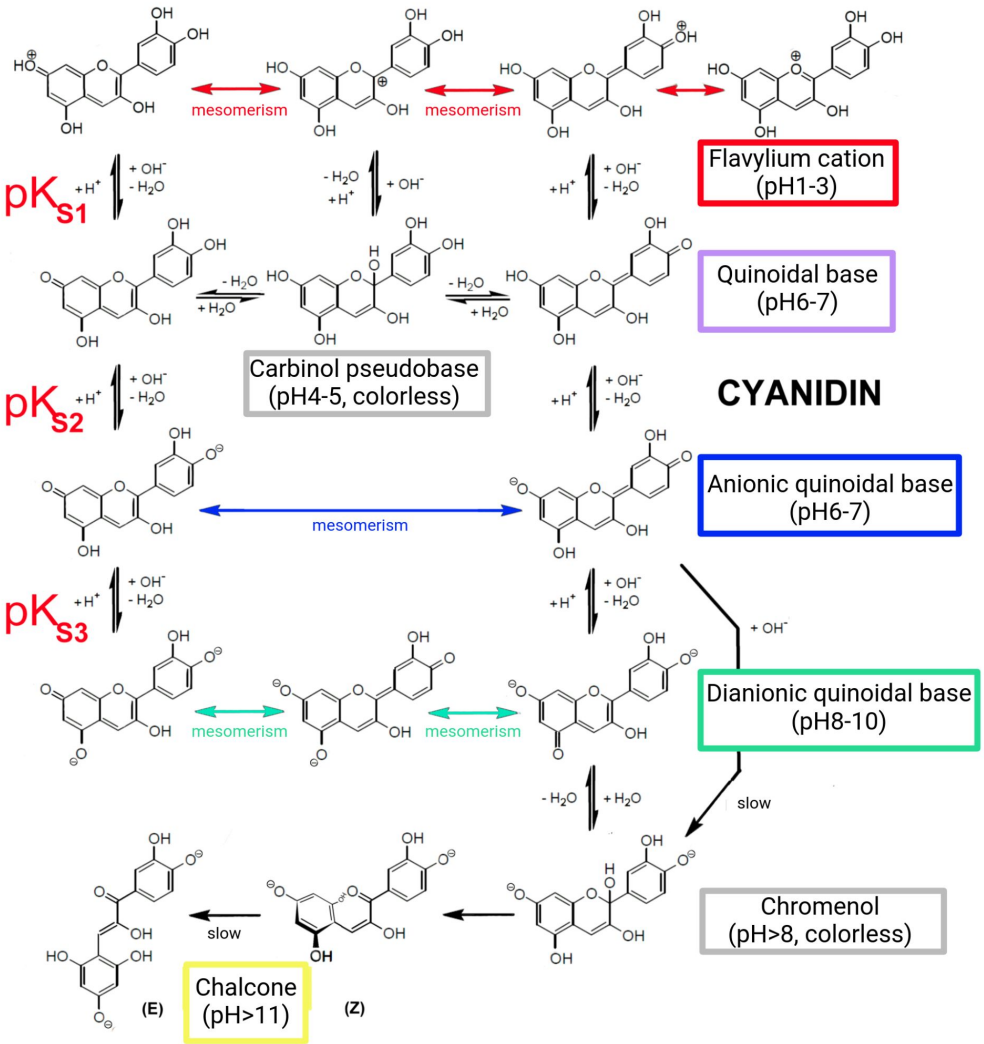
Hald, 2019: 10.1021/acs.jafc.8b06260

Anthocyanins

- Anthocyanins are orange, red, cyan, purple, or blue pigments
- Coloration of flowers and fruits to attract pollinators and seed dispersers
- Pigmentation in response to stress (high light, nitrogen starvation, cold/drought stress, salt stress)
- Complex decoration & modification with glycosylation, acylation, methylation



pH dependence of anthocyanin colors



Modified from https://commons.wikimedia.org/wiki/File:Cyanidin_-_Farbstoff_pH-Abhaengigkeit.png

Proanthocyanidins

- Oxidized proanthocyanidins are responsible for seed coat pigmentation

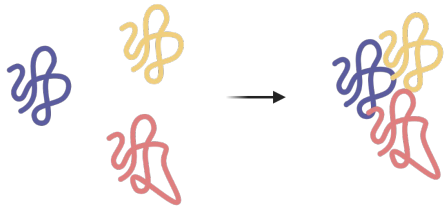
- PAs can influence taste and structure of food/drinks

André Karwath, CC BY-SA 2.5

- Increased PA biosynthesis in cassava resulted in two-spotted spider mite resistance

Neil Palmer (CIAT)

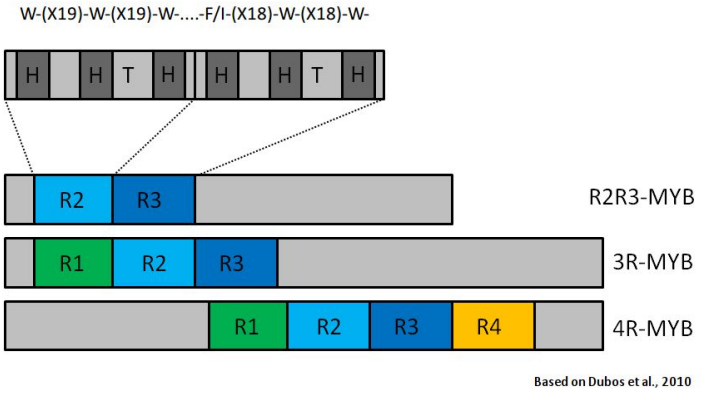
- Effect on protein aggregation and membrane disruption makes PAs antifeedants

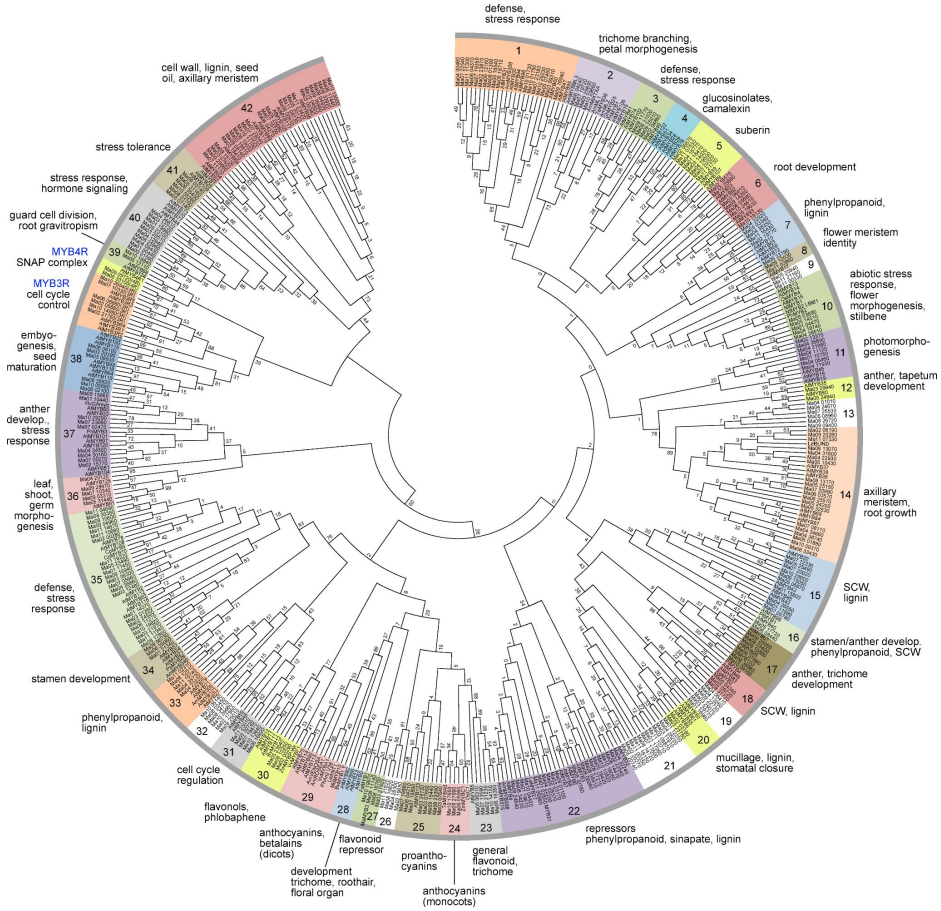


Gagne et al., 2009: 10.1016/j.plaphy.2008.12.004
Chen et al., 2022: 10.3389/fpls.2022.994866
Güngör et al., 2021: 10.1111/nph.16896

MYBs

- MYB (myeloblastosis) is one of the largest TF families in plants
- Characterized by repeats (R)
- R2R3-MYBs are particularly important in plants
- Involved in numerous functions in plants

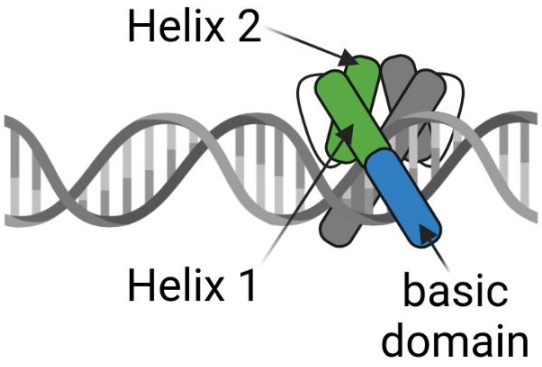


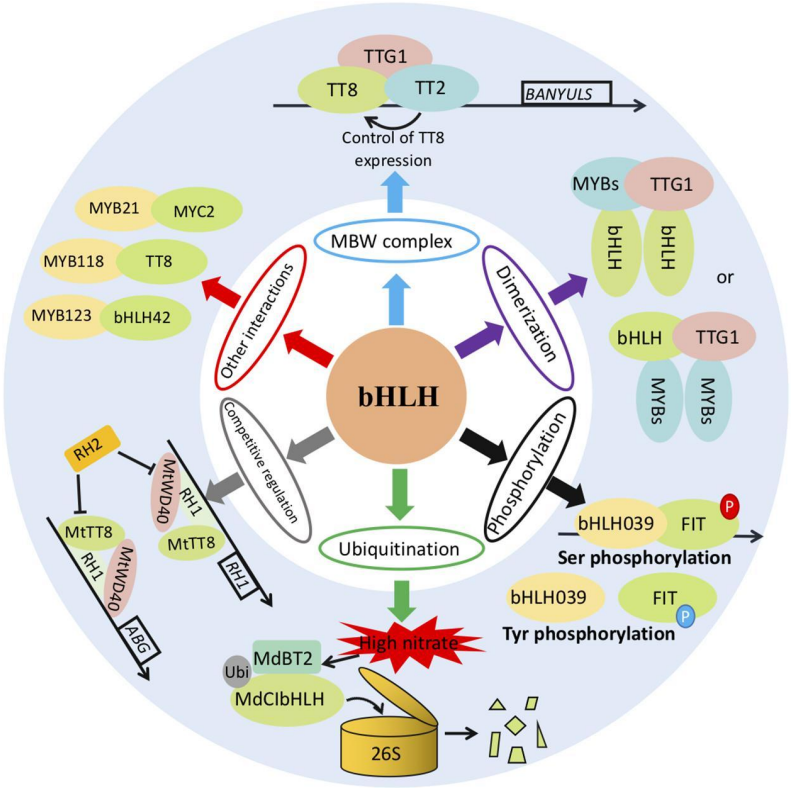


Pucker et al., 2020: 10.1371/journal.pone.0239275
Dubos et al., 2010: 10.1016/j.tplants.2010.06.005

bHLHs

- bHLH (basic Helix-Loop-Helix) transcription factors form a large family in plants
- Transcriptional activation in cooperation with MYBs and independently
- bHLH transcription factors operate often in dimers

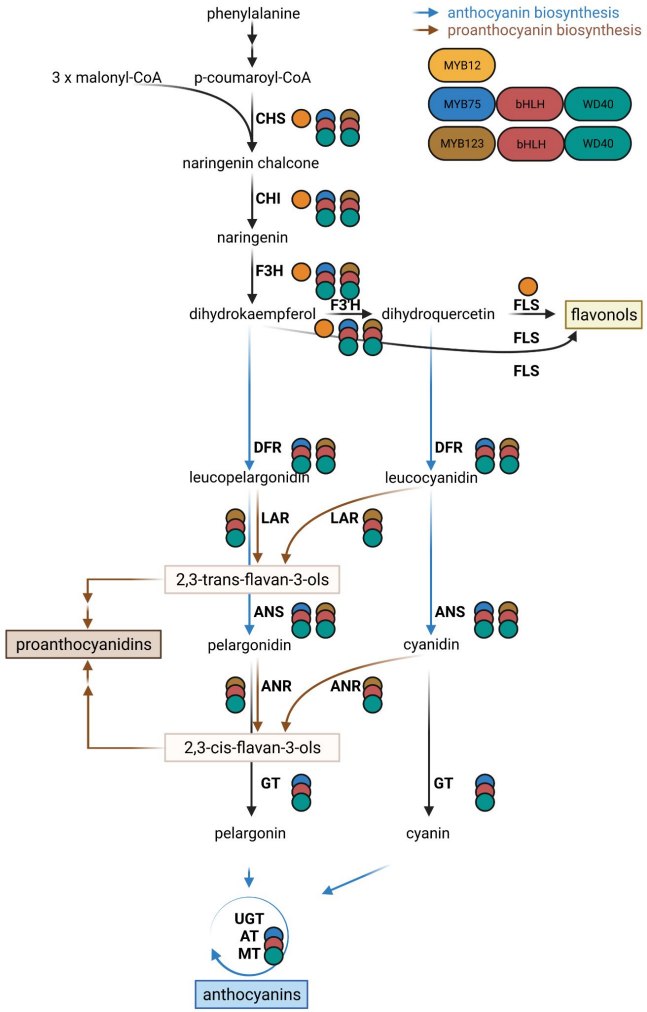




Qian et al., 2021: 10.3389/fpls.2021.677611

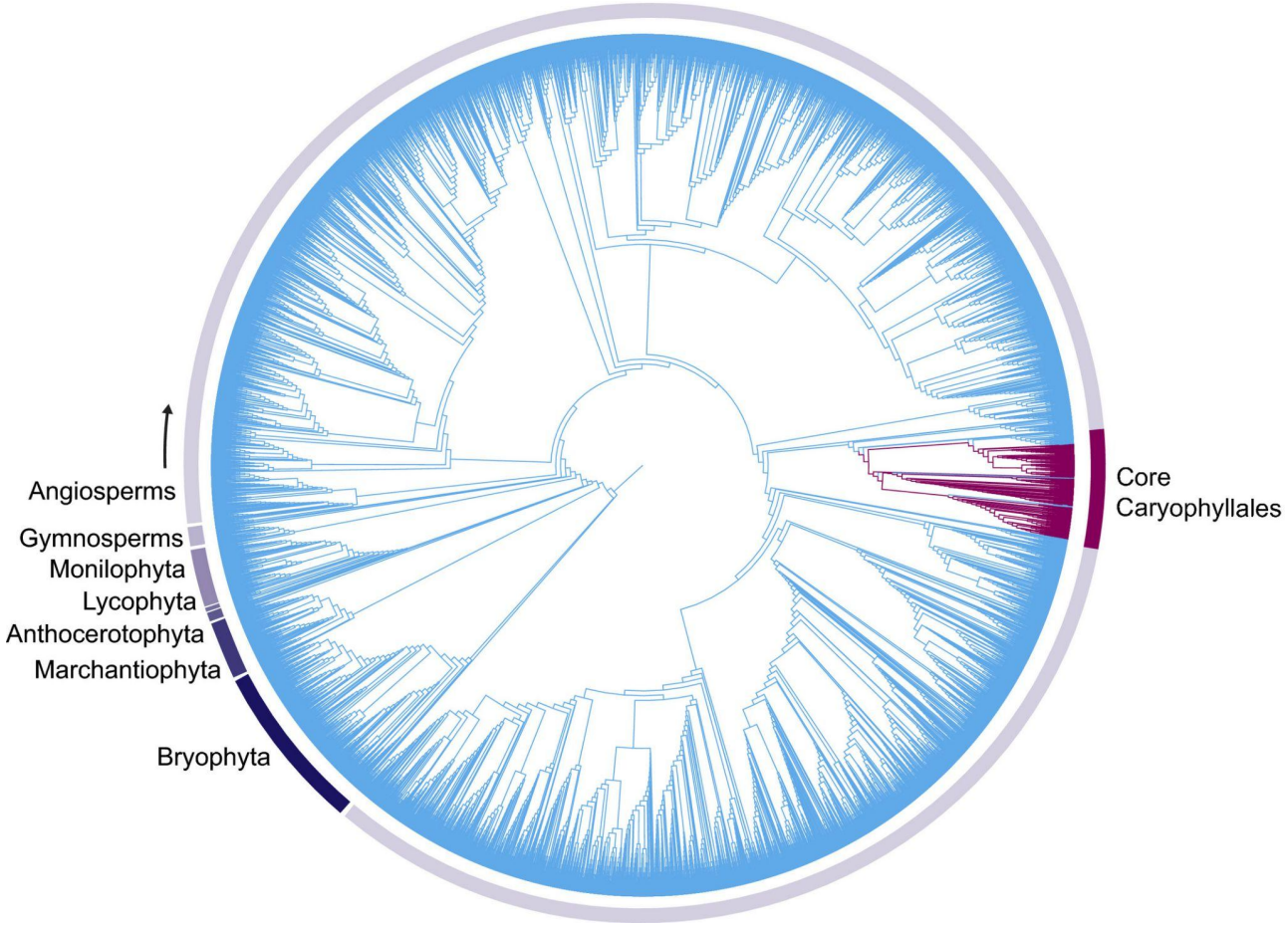
Transcriptional regulation of the flavonoid biosynthesis

- Flavonols:
  - MYB11/12/111 (subgroup 7)
  - MYB21/24/57 (subgroup 19)
- Anthocyanins: MBW complexes
  - MBW = MYB + bHLH + WD40
  - MYB crucial for specificity
  - MYB75/90/113/114
- Proanthocyanidins: MBW complexes
  - MYB123



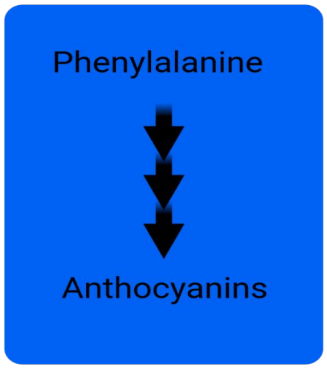
Complex pigment evolution in the Caryophyllales

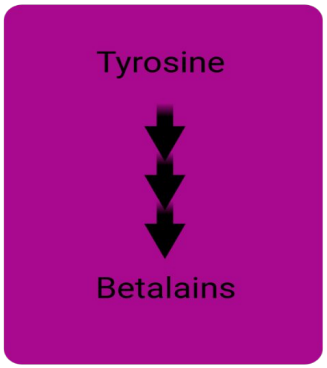
Betalains in the Caryophyllales

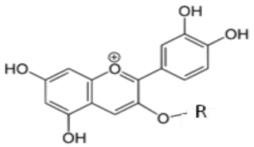


Timoneda *et al*., 2019: 10.1111/nph.15980

Functional redundancy of anthocyanins and betalains



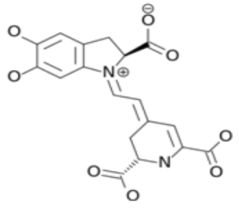




Anthocyanin

Antirrhinum majus

Mirabilis jalapa



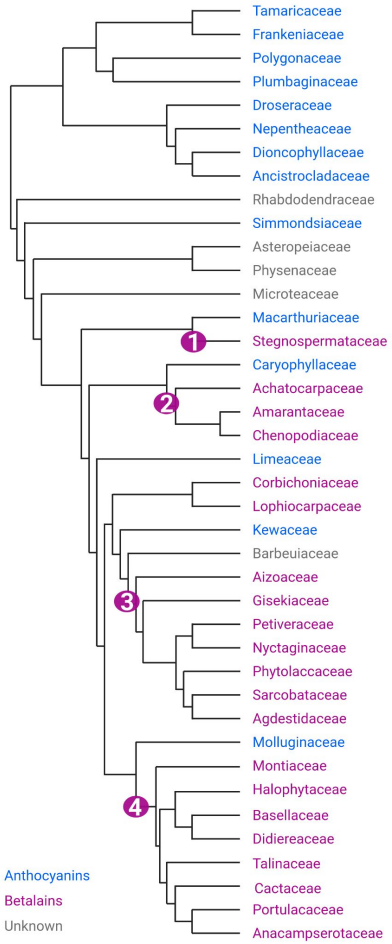
Betalain

Anthocyanin color range

Betalain color range

Brockington *et al.*, 2011: 10.1111/j.1469-8137.2011.03687.x

Complex pigment evolution in Caryophyllales



Stegnosperma cubense

Amaranthus cruentus

Mirabilis multiflora

Echinocereus pentalophus

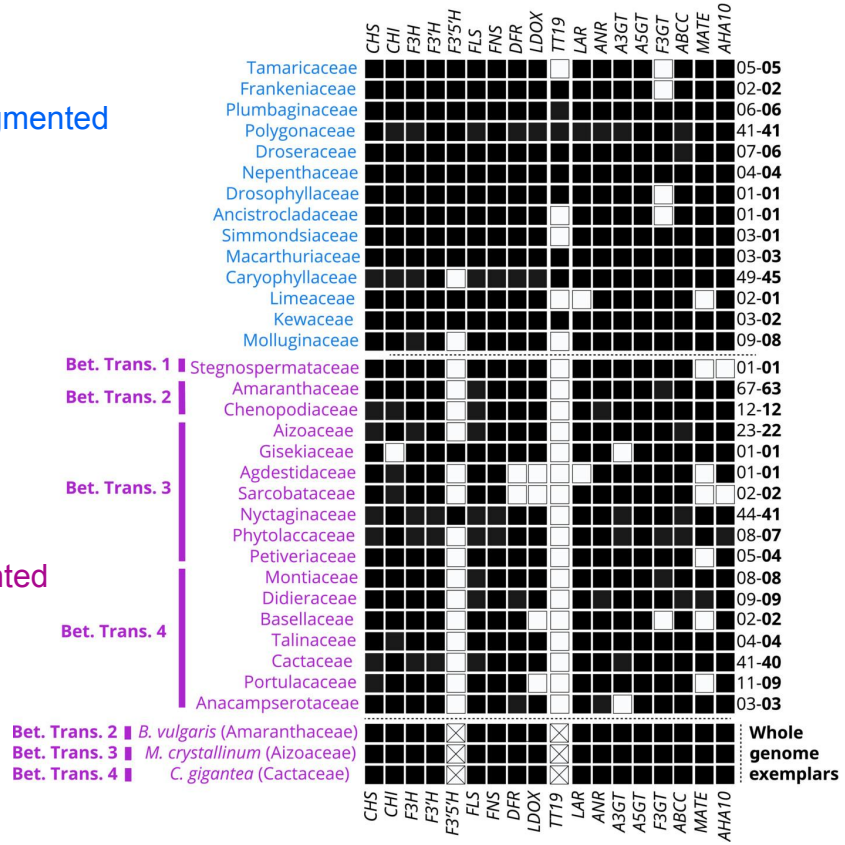
- At least four independent origins of betalain biosynthesis
- Mutual exclusion: anthocyanins and betalains were never observed in same (natural) plants
- Functional redundancy of both pigments

Timoneda *et al.*, 2019: 10.1111/nph.15980
Sheehan *et al.*, 2020: 10.1111/nph.16089
[1] Dick Culbert [2] Stan Shebs [3] Emöke Denes

Are anthocyanin biosynthesis genes missing in betalain-pigmented species?

Families of anthocyanin-pigmented species

Families of betalain-pigmented species

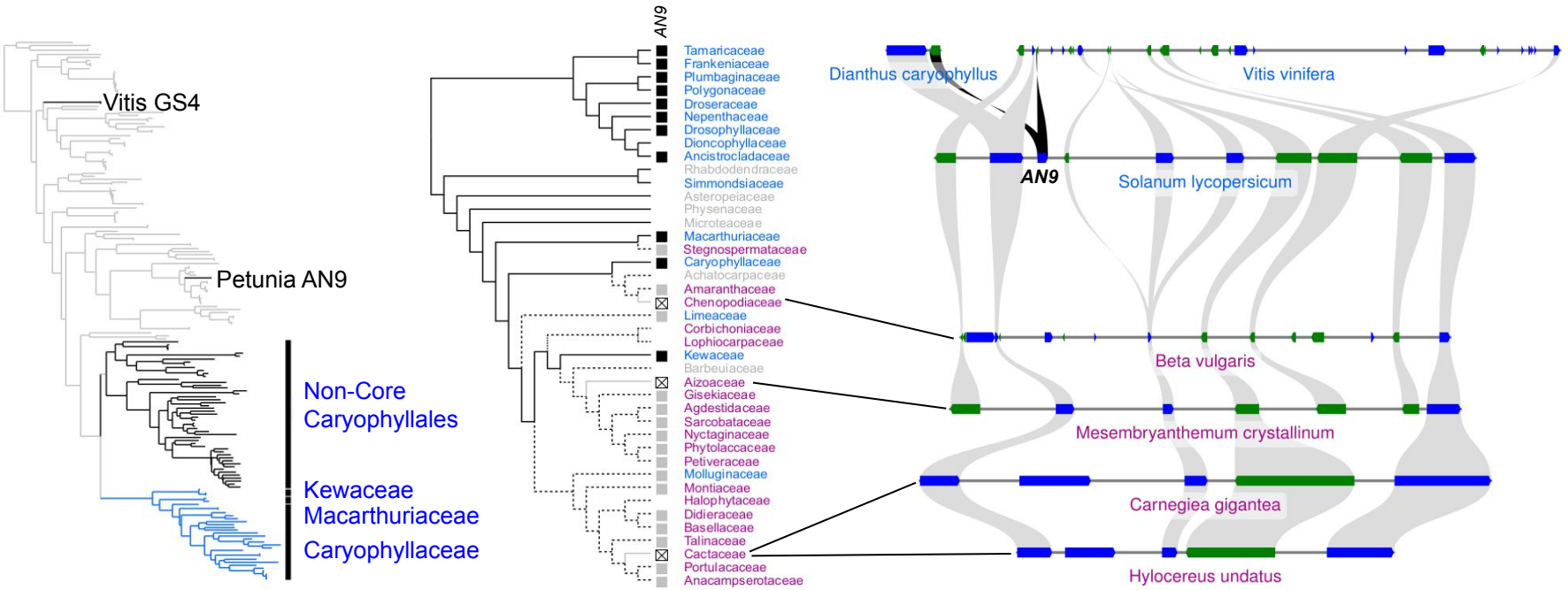


- Ratio of datasets per family showing a certain gene
- Anthocyanin biosynthesis genes are present throughout the Caryophyllales
- Lack of detection in some families is caused by incomplete datasets

Pucker *et al.*, 2023: 10.1111/nph.19341

Lack of *arGST* prevents anthocyanin accumulation

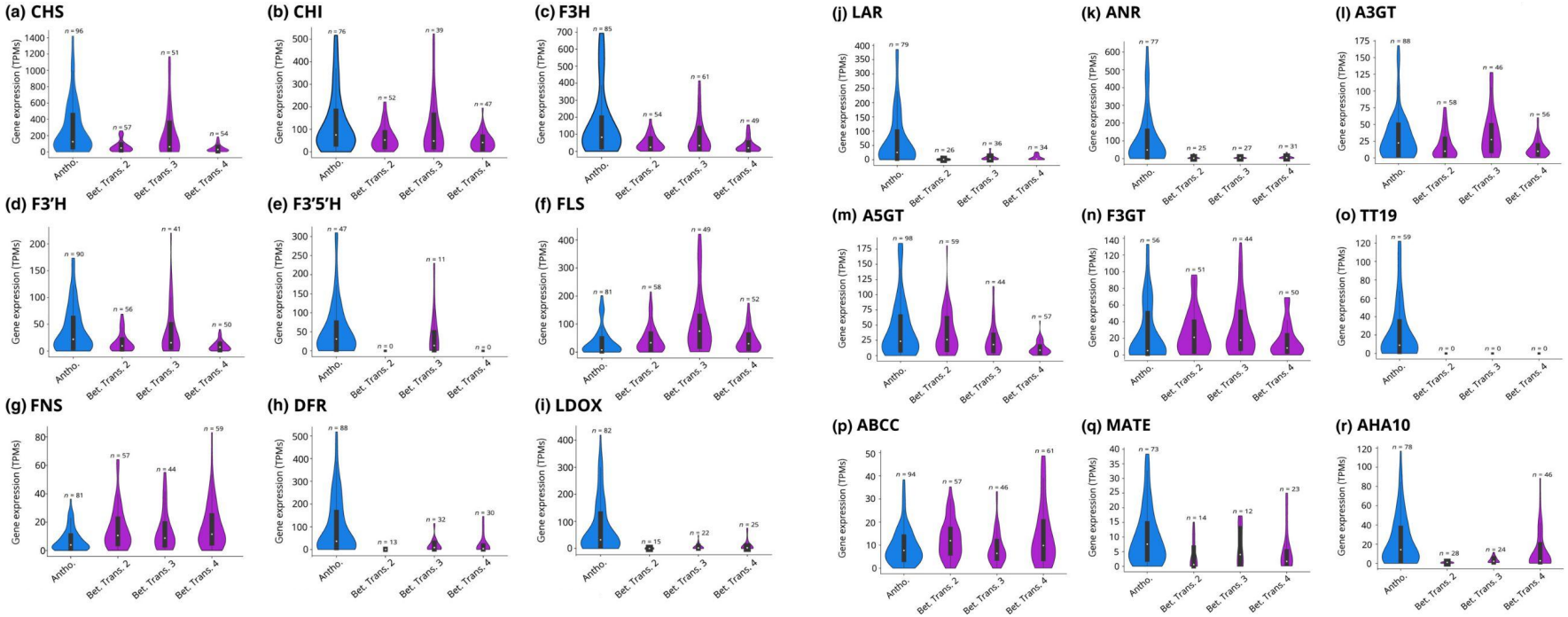
arGST = AN9, TT19, VvGS4, BZ1



Pucker *et al.*, 2022: 10.1101/2022.10.19.512958
Pucker *et al.*, 2023: 10.1111/nph.19341

Transcriptional activity of anthocyanin biosynthesis

- Anthocyanin biosynthesis genes DFR and ANS show very low transcript abundance in betalain-pigmented Caryophyllales species



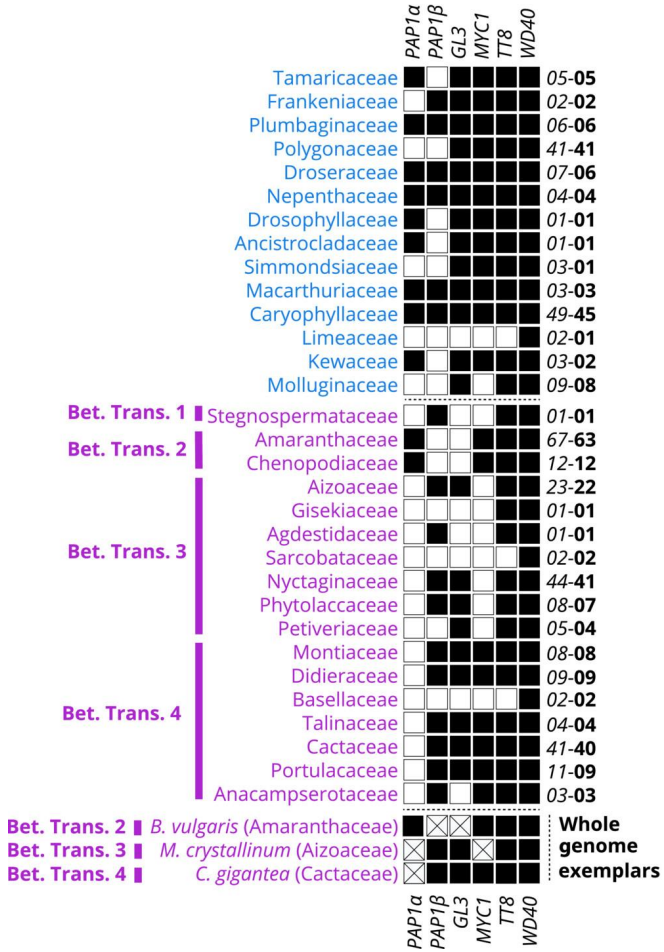
Pucker *et al.*, 2023: 10.1111/nph.19341

What about anthocyanin transcription factors?

- Anthocyanin biosynthesis regulated by MBW complex:
  - **M**YB (PAP1)
  - **b**HLH (GL3, MYC1, TT8)
  - **W**D40

- PAP = Production of Anthocyanin Pigmentation

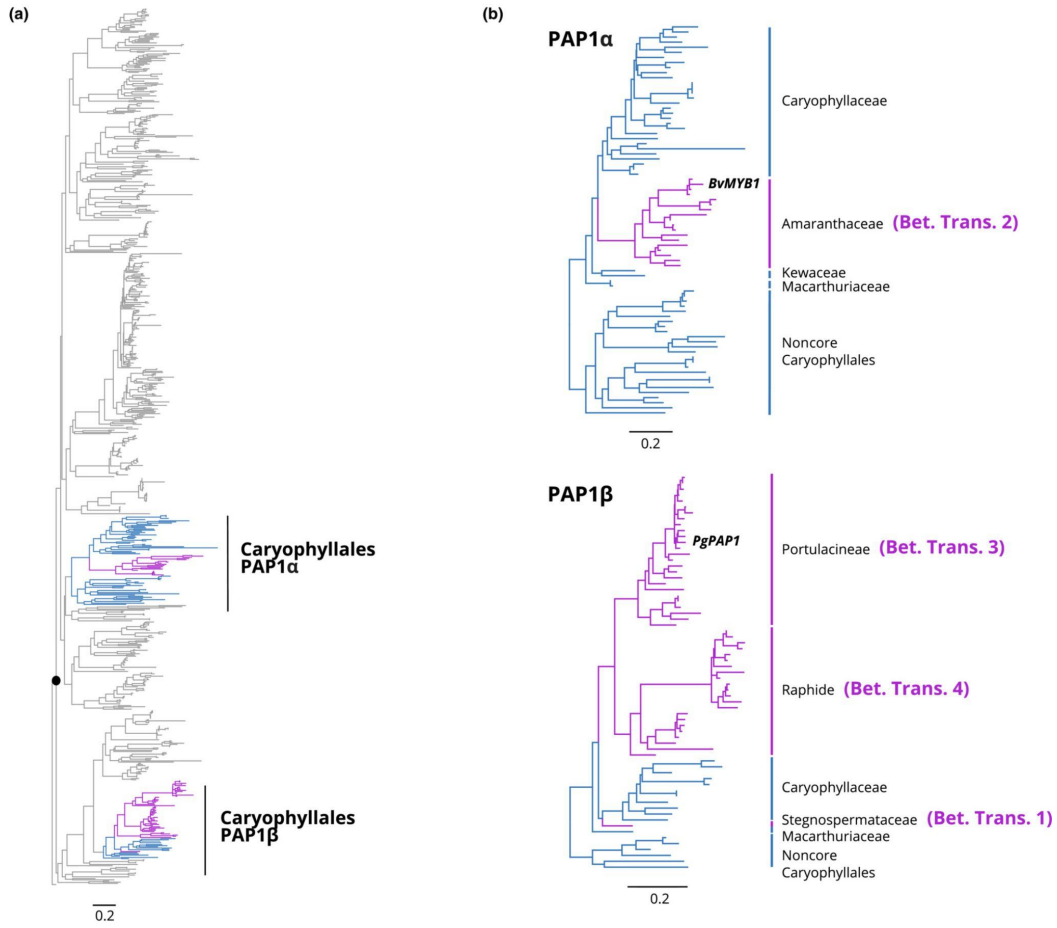
- All MBW complex components are generally present in anthocyanin-pigmented and betalain-pigmented Caryophyllales



Pucker *et al.*, 2023: 10.1111/nph.19341

Deep duplication of PAP1

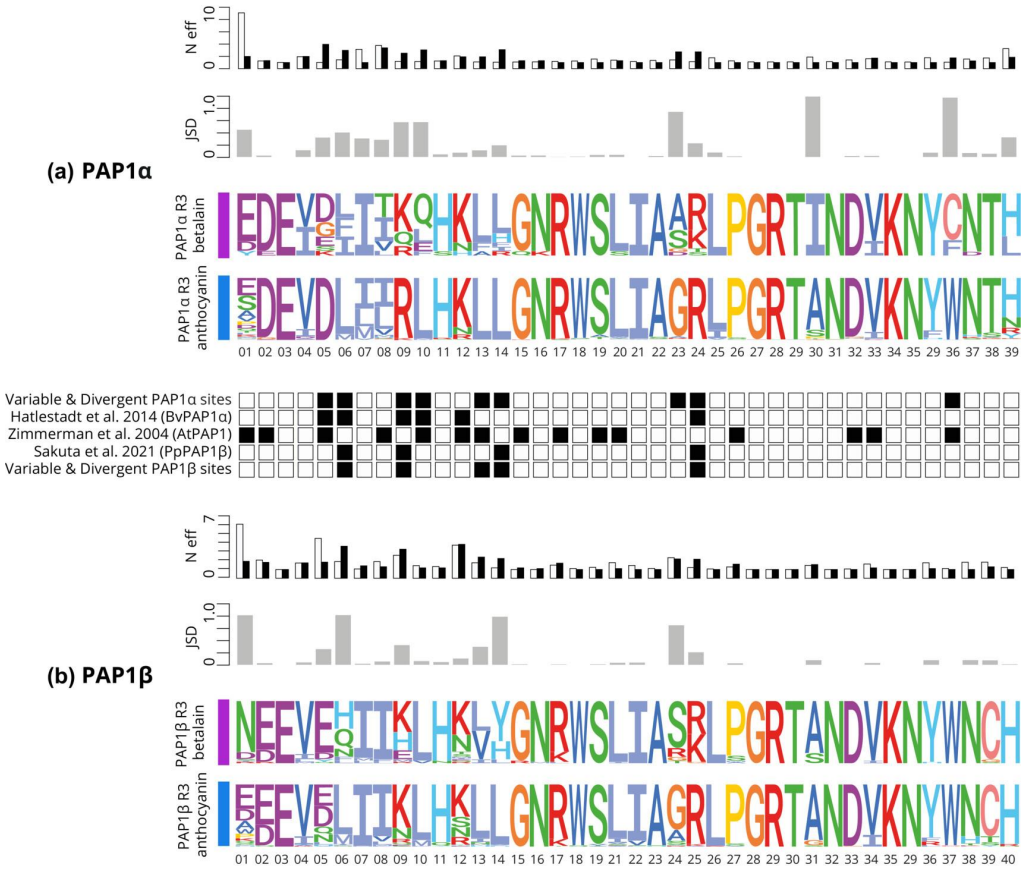
- Deep PAP1 duplication in land plants
- Both PAP1 lineages retained in Caryophyllales
- Differential loss of PAP1 in Caryophyllales (usually one copy per species)



Pucker *et al.*, 2023: 10.1111/nph.19341

Loss of MYB-bHLH interaction motif

- MYB and bHLH need to interact to form MBW complex
- PAP1 in the betalain-pigmented Caryophyllales lost this motif
- Independent loss of interaction motif in different betalain lineages



Pucker *et al.*, 2023: 10.1111/nph.19341

Summary

- Visible flavonoids - anthocyanins
- Branches of the flavonoid biosynthesis
- Transcriptional regulation of the flavonoid biosynthesis
- Complex pigment evolution in the Caryophyllales

Time for questions!

Questions

1. How many flavonoids are known?
2. What are the different branches of the flavonoid biosynthesis?
3. How are anthocyanins modified?
4. How can we know that early branches are basis for evolution of later branches?
5. What are important anthocyanin properties and functions?
6. Which transcription factor families are highly relevant for flavonoid biosynthesis regulation?
7. What do you know about the complex pigment evolution in the Caryophyllales?