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@PuckerLab

Prof. Dr. Boas Pucker

PBPM-BP-05

Availability of slides

- All materials are freely available (CC BY) - after the lectures:
  - eCampus: PBPM0 - Plant Biochemistry, Physiology and Molecular Biology (LEC)
  - GitHub: https://github.com/bpucker/teaching/PBPM

- Questions: Feel free to ask at any time

- Feedback, comments, or questions: pucker[a]uni-bonn.de

Repetition

- Translation
- Lipids: membrane vs. storage
- Carbohydrates: sugar, starch, cellulose
- Nitrate assimilation and metabolism

What are specialized plant metabolites?

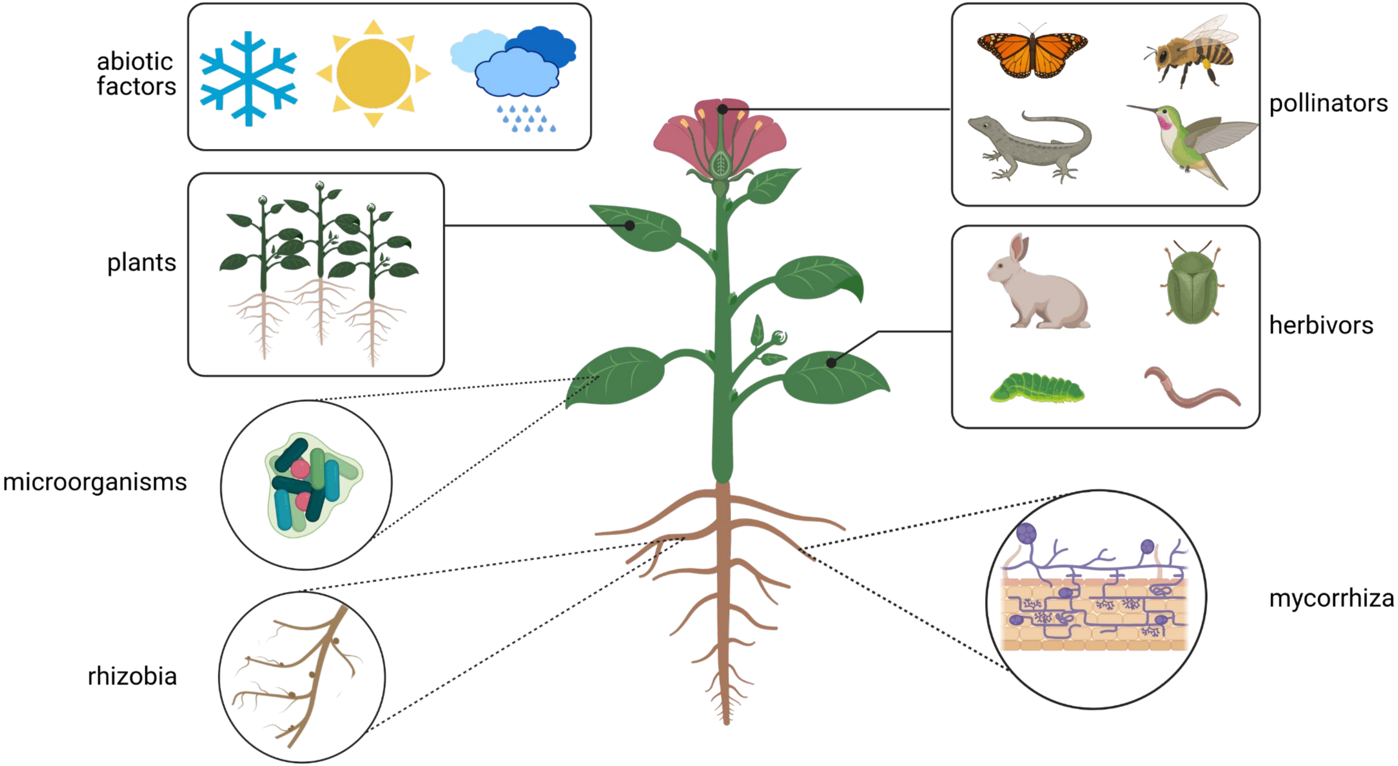
- Definition: metabolites conferring evolutionary advantage, but not immediately required for survival

- *Papaver somniferum*: morphine (pain)

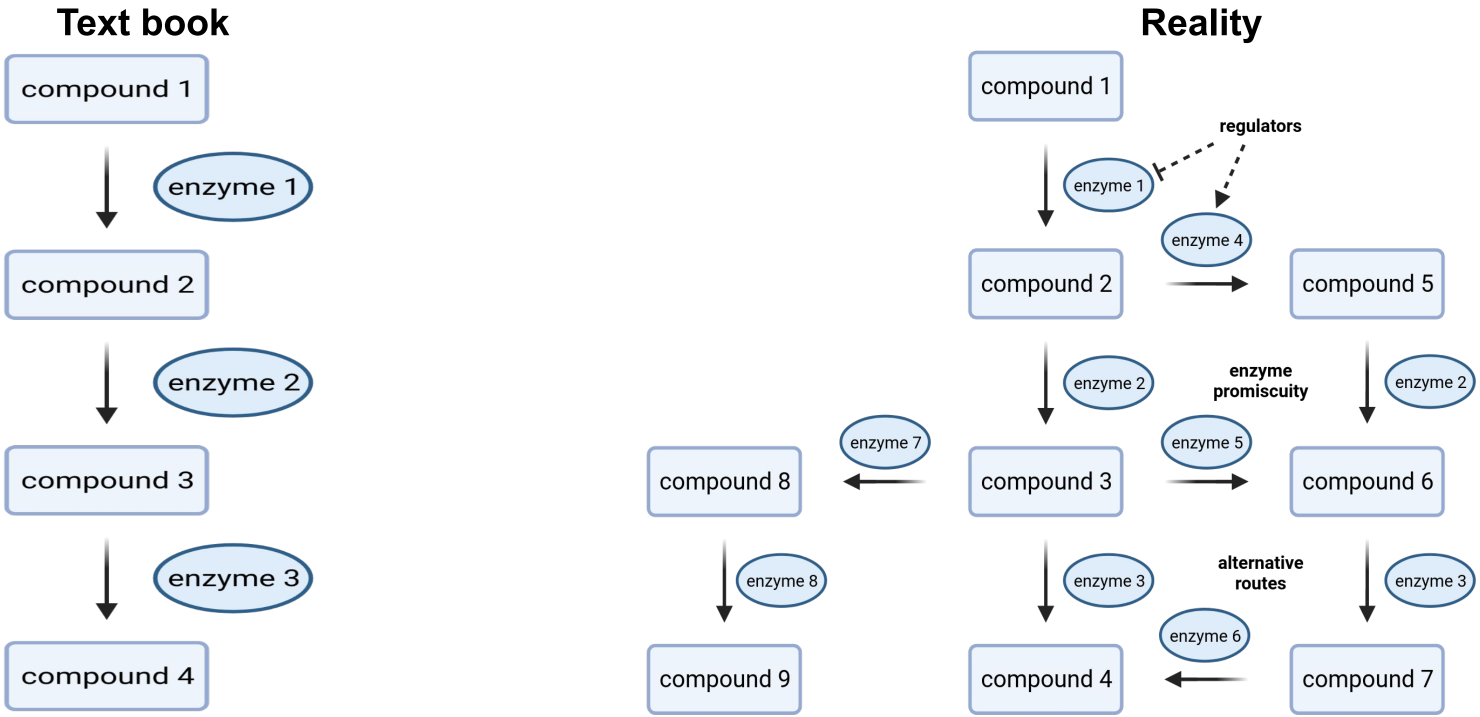
- *Digitalis purpurea*: digoxin (congestive heart failure)

- *Taxus brevifolia*: paclitaxel (cancer)

Why do plants produce specialized metabolites?

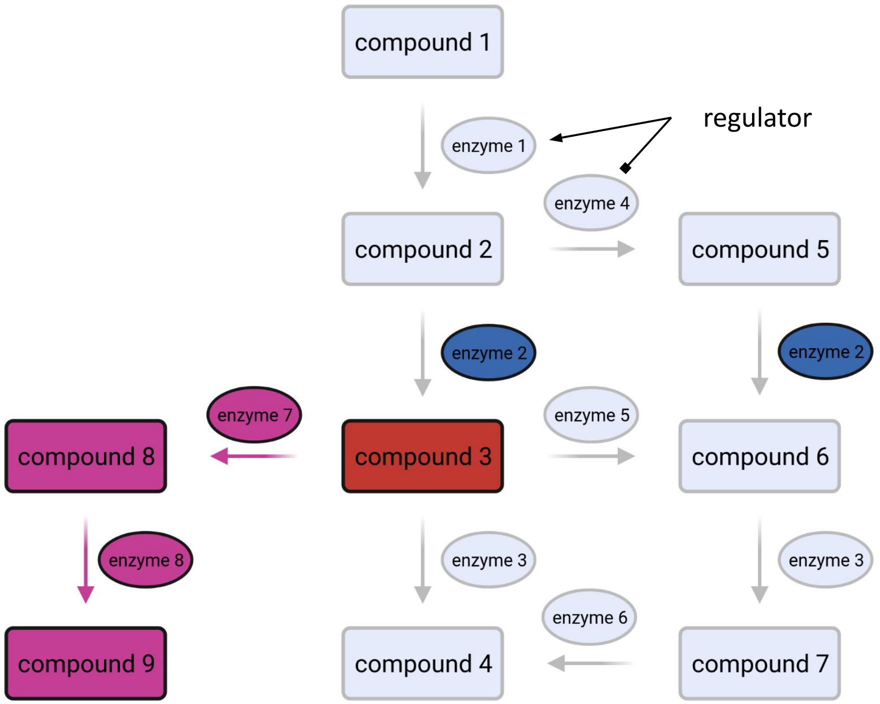


How are specialized metabolites produced?



Which factors cause network complexity?

- Metabolic networks are characterized by hubs (central intermediates)
- Many enzymes show **promiscuity** i.e. catalyze different reactions
- Branches could be considered linear pathways
- Edge (reactions); nodes (compounds)



Pigments as colorful biosynthesis models

Chlorophyll

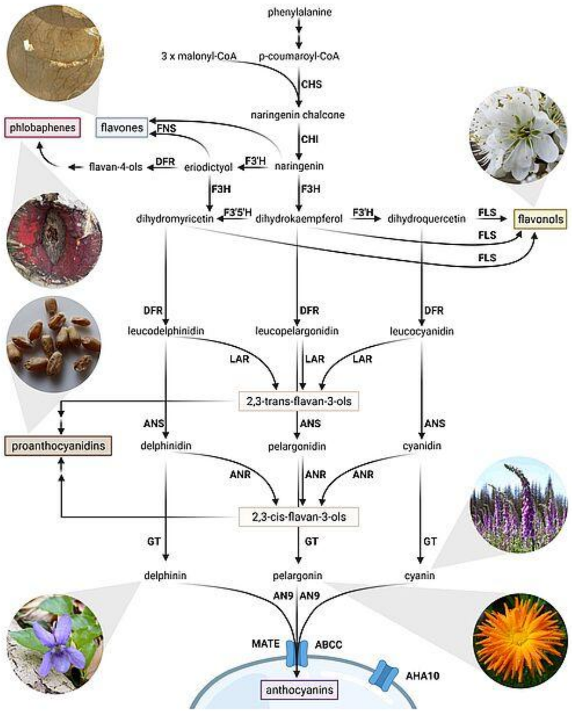
Carotenoids

Anthocyanins

Betalains

Flavonoid biosynthesis branches

- Flavones
- Flavonols
- Proanthocyanidins
- Anthocyanins



Flavones

- Examples: Apigenin, luteolin, baicalein, chrysin, tangeretin
- Sources: parsley, celery, chamomile, citrus peels, thyme, and mint
- Biological roles: UV protection, pigmentation (pale yellow), defense against pathogens and herbivores
- Generally more chemically stable than anthocyanins
- Often occur as glycosides (bound to sugars)

Feng et al., 2023: 10.1038/s42003-023-05044-1

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Flavonols

- Structurally similar to flavones with extra hydroxyl group at position 3
- Examples: quercetin, kaempferol, myricetin, isorhamnetin, fisetin
- Sources: onions, kale, apples, tea, broccoli, grapes, and berries
- Biological roles: protect against UV, pale yellow pigmentation, involved in plant defense mechanisms
- Chemical Features: occur mostly as glycosides, widely distributed

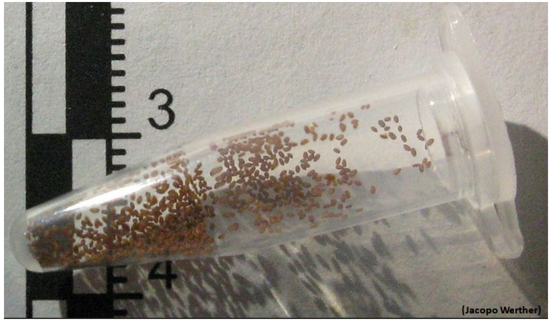
Proanthocyanidins

- Oligomeric or polymeric flavonoids composed of flavan-3-ol units (mainly catechin and epicatechin)

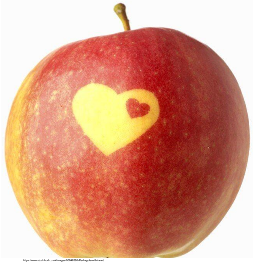
- Sources: grape seeds and skins, cocoa, apples, cranberries, blueberries, pine bark

- Biological roles: defense against herbivores and pathogens, contribute to astringency and color stability in fruits

- Chemical Features: colorless, often occur as polymers, stability influenced by pH, temperature, and degree of polymerization



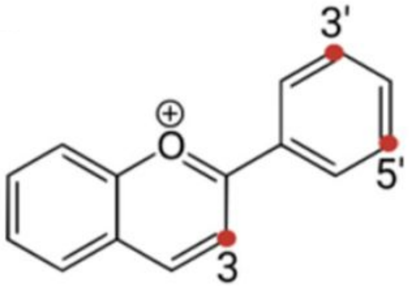
Anthocyanins - colorful pigments as model



https://doi.org/10.1016/j.ymben.2018.06.004

Anthocyanins

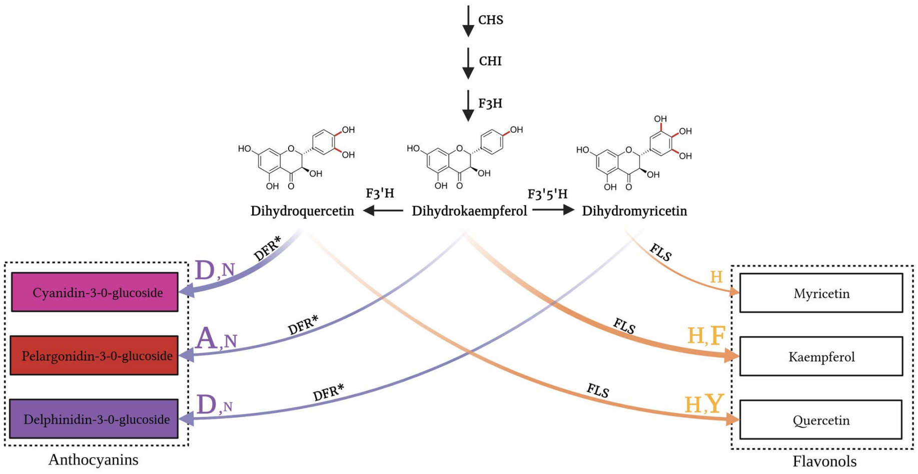
- Water-soluble flavonoid pigments responsible for the red, purple, and blue colors in many plants, fruits, and vegetables
- Glycosides of anthocyanidins — meaning an anthocyanidin molecule bound to one or more sugar molecules
- Based on the flavylium ion ($C_{15}H_{11}O^{+}$)
- Major anthocyanidins: Cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin



Pucker & Selmar, 2022: 10.3390/plants11070963

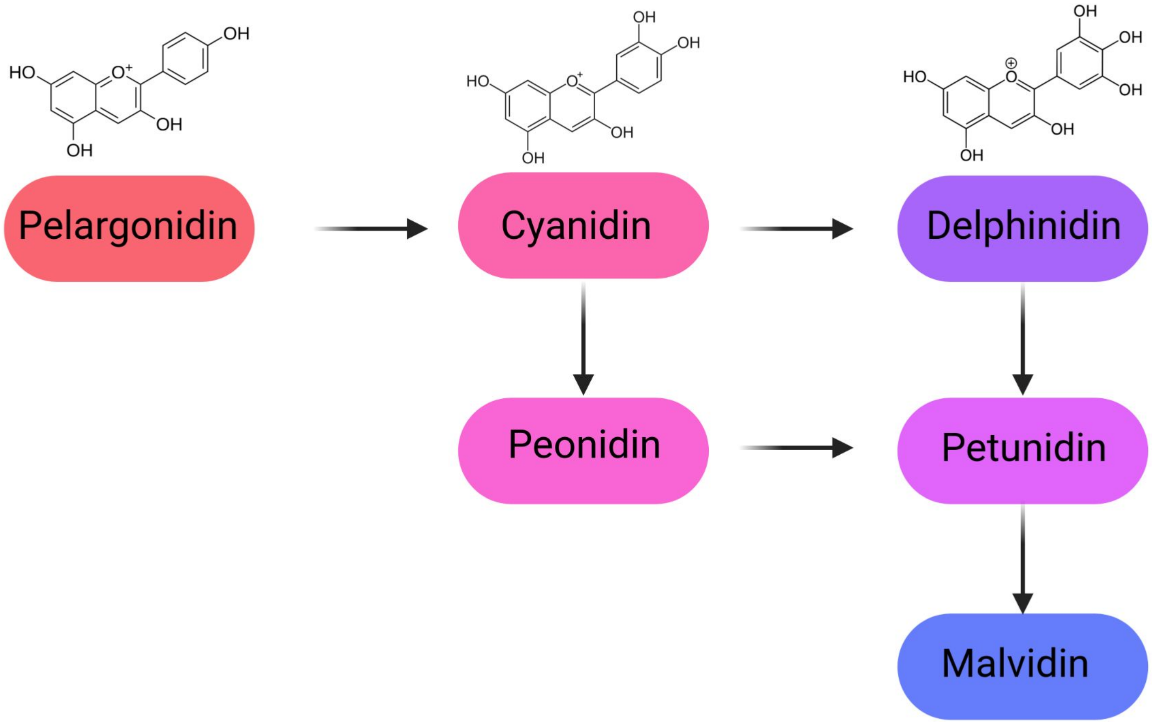
Hydroxylation pattern

- One, two, or three hydroxy groups
- Color and other properties depend on hydroxylation pattern
- F3’H and F3’5’H are responsible for flavonoid hydroxylation



Choudhary & Pucker, 2024: 10.1371/journal.pone.0305837

Biochemical basis of color



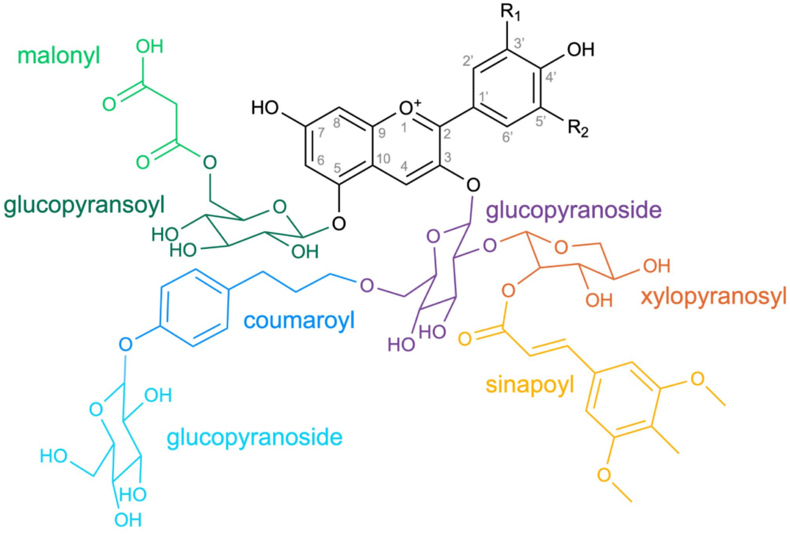
https://github.com/bpucker/FlavonoidFriday

Decoration of anthocyanins

- Anthocyanidins are turned into anthocyanins by addition of sugar moieties

- Further modification by addition of sugar moieties, acyl, or methyl groups

- Modification often at 3, 5, 3’, and 5’ position



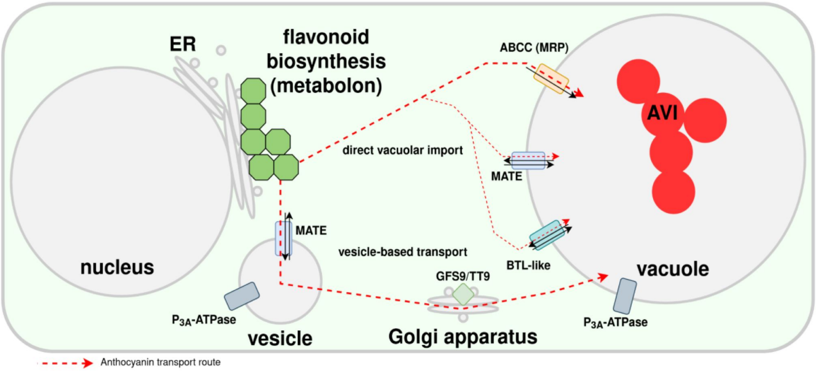
Grünig et al., 2025: 10.20944/preprints202408.2272.v1

Anthocyanin transport

- Anthocyanins accumulate in the centrale vacuole

- Postulated transport routes via vesicles or direct uptake across tonoplast

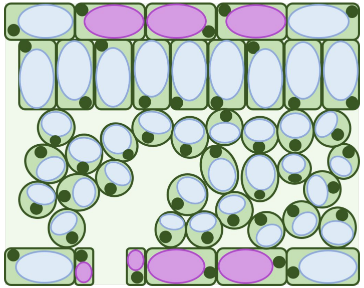
- Additional decoration in vacuole possible

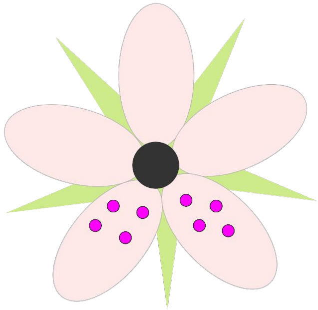


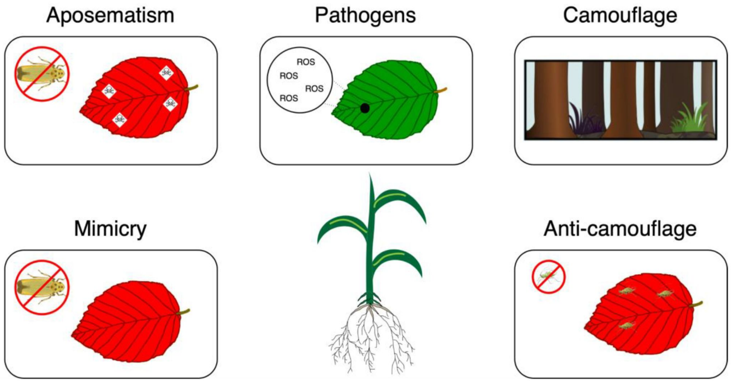
Grünig et al., 2025: 10.20944/preprints202408.2272.v1

Ecological functions of anthocyanins

- Attraction of pollinators
- Attraction of seed dispersers
- Protection of plant leaves against intense sun light
- Functions in defense (camouflage, mimicry, anti-camouflage, …)



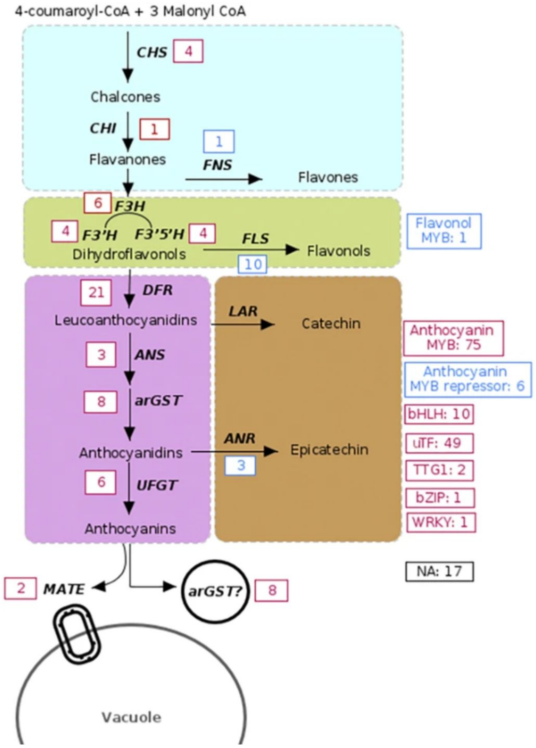


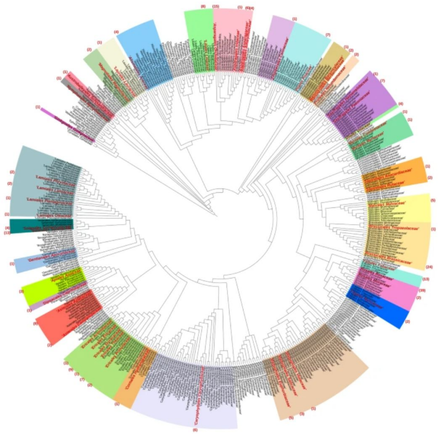


Grünig et al., 2025: 10.20944/preprints202408.2272.v1

Anthocyanins as a model system

- Easily visible phenotype (color)
- Extensively studied for evolutionary questions
- >200 studies explored the molecular basis of anthocyanin loss

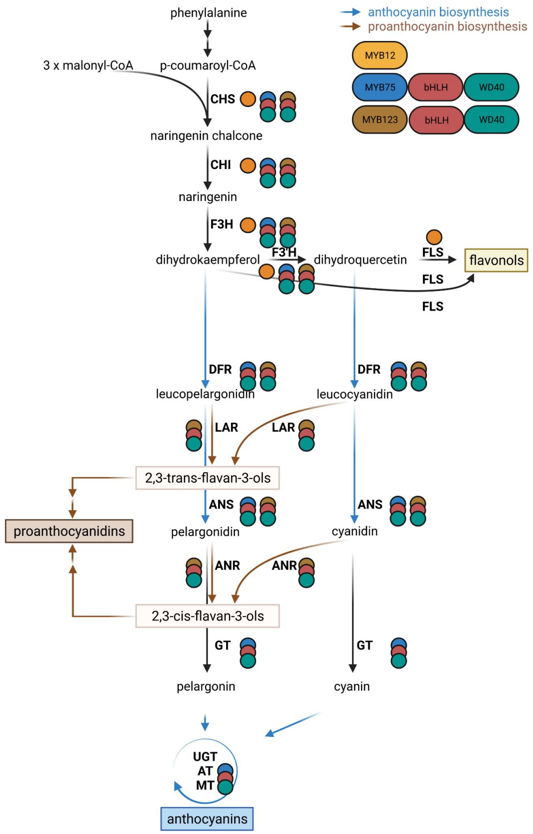




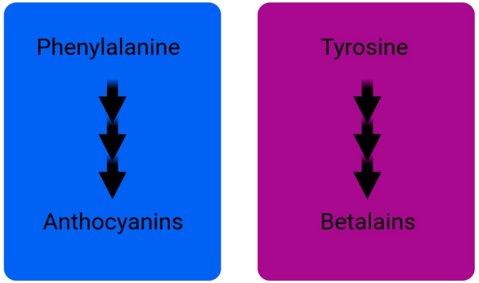
Marin-Recinos & Pucker, 2024: 10.1186/s12870-024-05316-w

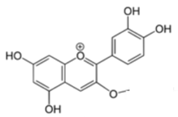
Flavonoid biosynthesis mutants

- Example 1: *fls* or *myb12*
  - Flavonols (-)
  - Anthocyanins (+)
  - Proanthocyanidins (+)
- Example 2: *chs*, *chi*, or *f3h*
  - Flavonols (-)
  - Anthocyanins (-)
  - Proanthocyanidins (-)
- Example 3: *bhlh*, *wd40*, *dfr*
  - Flavonols (+)
  - Anthocyanins (-)
  - Proanthocyanidins (-)



Betalains

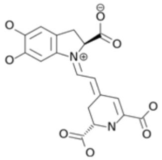




Anthocyanin

Antirrhinum majus

Mirabilis jalapa



Betalain

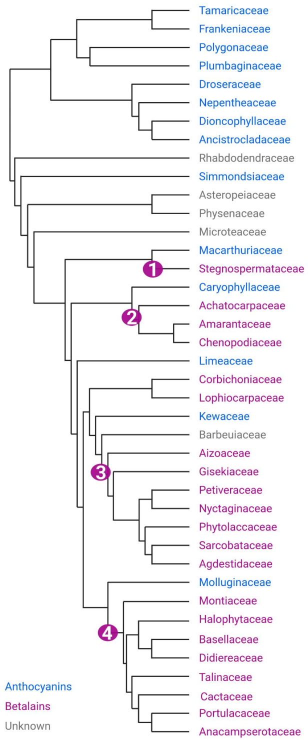
Anthocyanin color range

Betalain color range

- Betalains are based on tyrosine
- Colors range from pink to yellow
- Same flower color can be caused by anthocyanins or betalains

Timoneda *et al.*, 2019: 10.1111/nph.15980
Sheehan *et al.*, 2020: 10.1111/nph.16089

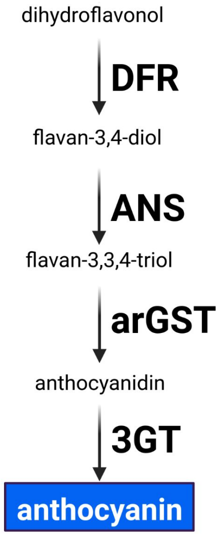
Complex pigmentation evolution in Caryophyllales

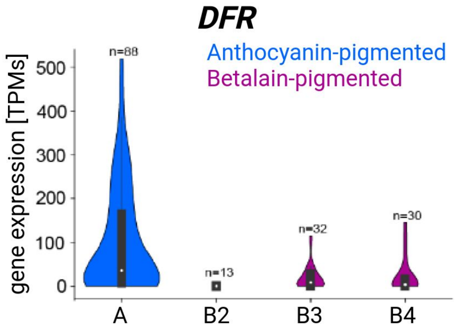


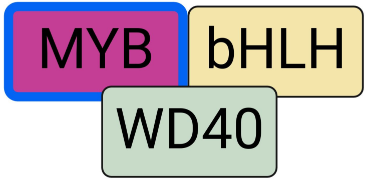
- Anthocyanin loss in entire plant families
- Anthocyanins and betalains appear functionally redundant
- Mutual exclusion: anthocyanins and betalains were never observed in same plants

Timoneda *et al.*, 2019: 10.1111/nph.15980
Sheehan *et al.*, 2020: 10.1111/nph.16089

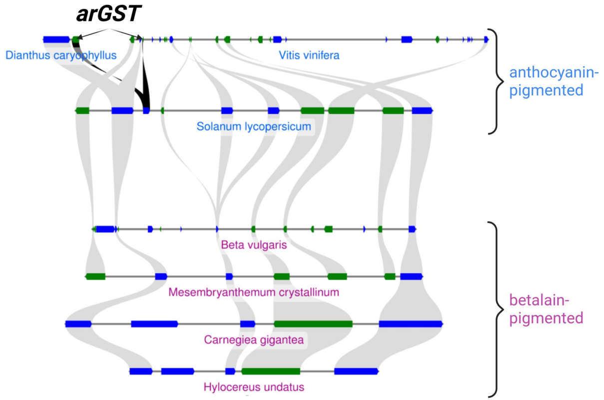
Anthocyanin loss in betalain-pigmented Caryophyllales







- Low transcriptional activity of central anthocyanin genes *DFR* and *ANS*
- Neofunctionalization of anthocyanin activating MYB transcription factor
- Loss of *arGST* genes in betalain-pigmented families



Pucker *et al.*, 2024: 10.1111/nph.19341

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Terpenoids

- Terpenoids are large & diverse class of naturally occurring compounds derived from isoprene units (C_5H_8)
- Isoprenoids due to repeating isoprene (2-methyl-1,3-butadiene) units
- Isoprene rule (C_5 rule): constructed from isoprene (C_5H_8) units joined in a “head-to-tail” manner
- General formula: (C_5H_8)□

Type	No. of Isoprene Units	Carbon Atoms
Hemiterpenoids	1	C_5
Monoterpenoids	2	C_{10}
Sesquiterpenoids	3	C_{15}
Diterpenoids	4	C_{20}
Sesterterpenoids	5	C_{25}
Triterpenoids	6	C_{30}
Tetraterpenoids	8	C_{40}
Polyterpenoids	Many	Variable

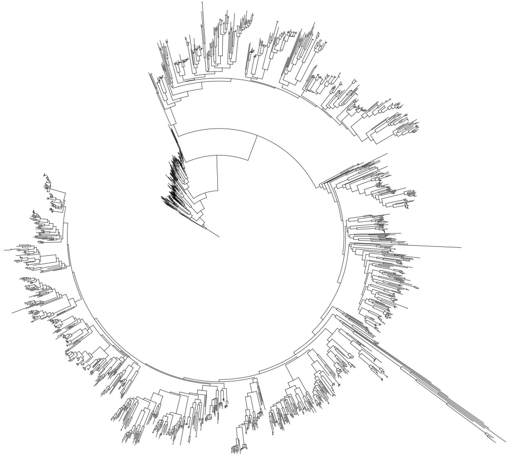
Terpene synthases

- Convert simple prenyl diphosphate precursors into enormous diversity of plant terpenes
- Single TPS can generate multiple terpenes through carbocation rearrangements
- Typically act by generating highly reactive carbocations, which undergo: ionization, cyclization, hydride/methyl shifts, rearrangements, termination by deprotonation or water capture
- Most TPSs require Mg^{2+} for substrate binding
- DDxxD and NSE/DTE motifs (bind metal ions and substrate diphosphates)
- RRX_8W motif in many monoterpene synthases (involved in isomerization/cyclization)

Jeroen S. Dickschat (Organic Chemistry & Biochemistry)

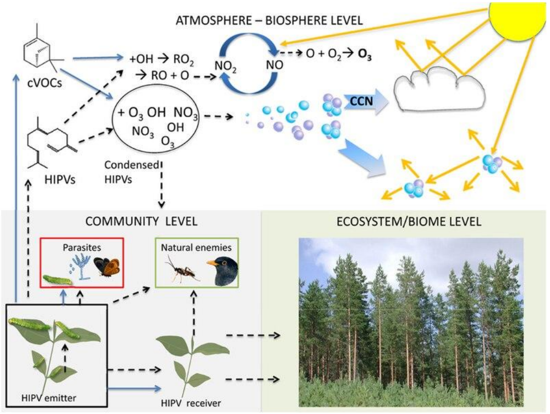
Evolution of chemical diversity

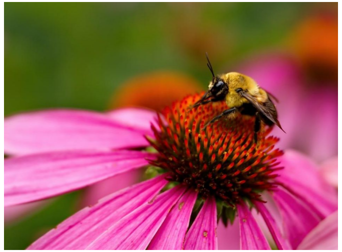
- Subtle mutations → dramatic product changes, underlying their evolvability
- Chemical diversity, enabling adaptation to ecological pressures
- Large, plant-specific gene families; especially expanded in angiosperms
- Derived from ancient bifunctional diterpene cyclases
- High duplication rates → neofunctionalization



Biological functions of terpenoids

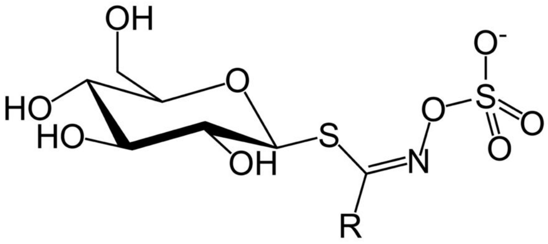
- Defense against herbivores & pathogens (e.g., volatile monoterpenes, phytoalexin diterpenes)
- Indirect defense (attract predators/parasitoids of herbivores)
- Abiotic stress tolerance (e.g., isoprene reduces oxidative stress)
- Pollinator attraction (floral scents)
- Species-specific chemical signatures used in plant–plant (e.g. allelopathy) or plant–microbe interactions





https://doi.org/10.3389/fpls.2013.00185
https://freerangestock.com/photos/151249/a-bee-on-a-flower.html

Glucosinolates



- Sulfur- and nitrogen-containing metabolites
  - β-D-glucopyranose, a sulfur-linked oxime, and a variable side chain (R-group)

- Occurrence: broccoli, cabbage, mustard, radish, cauliflower, and kale

- Enzymatic hydrolysis by myrosinase (activated by tissue damage)
- Biological Roles: defense compounds against insects and pathogens

- Examples:
  - Sinigrin ⟶ Allyl isothiocyanate (mustard oil)
  - Glucoraphanin ⟶ Sulforaphane (anticancer agent in broccoli)

Glucosinolate examples

- Sinigrin: a common aliphatic GSL; when hydrolyzed by myrosinase, it produces allyl isothiocyanate (“mustard oil” - pungency deters herbivores)
- *A. thaliana*: when plants are attacked by various insects (both generalists and specialists), they often upregulate aliphatic GSLs
- Diamondback moth (Plutella xylostella): a specialist herbivore on Brassicaceae, has evolved glucosinolate sulfatase (GSS) enzymes that desulfate GSLs in its gut, forming desulfoglucosinolates that myrosinase cannot hydrolyze — effectively “disarming” the mustard-oil bomb
- *Brassica juncea* (mustard): levels of GSLs and myrosinase decline in seedlings as cotyledons mature — matching optimal defense theory: young, valuable tissues are better defended

Mewis et al., 2006: 10.1016/j.phytochem.2006.09.004
Ratzka et al., 2002: 10.1073/pnas.172112899
Wallace & Eigenbrode, 2002: 10.1023/a:1017973005994

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Summary

- Specialized metabolites
- Flavonoids: flavonols, proanthocyanidins, anthocyanins
- Betalains
- Terpenoids
- Glucosinolates

Time for questions!

Questions

1. What are specialized metabolites?
2. What are functions of specialized metabolites?
3. What are the different branches of the flavonoid biosynthesis?
4. What are ecological functions of anthocyanins?
5. Which plants are pigmented by betalains?
6. What is the complex pigment evolution involving anthocyanins and betalains?
7. What are the characteristics of terpenoids?
8. What are the characteristics of glucosinolates?