

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](mailto:pucker[a]uni-bonn.de)



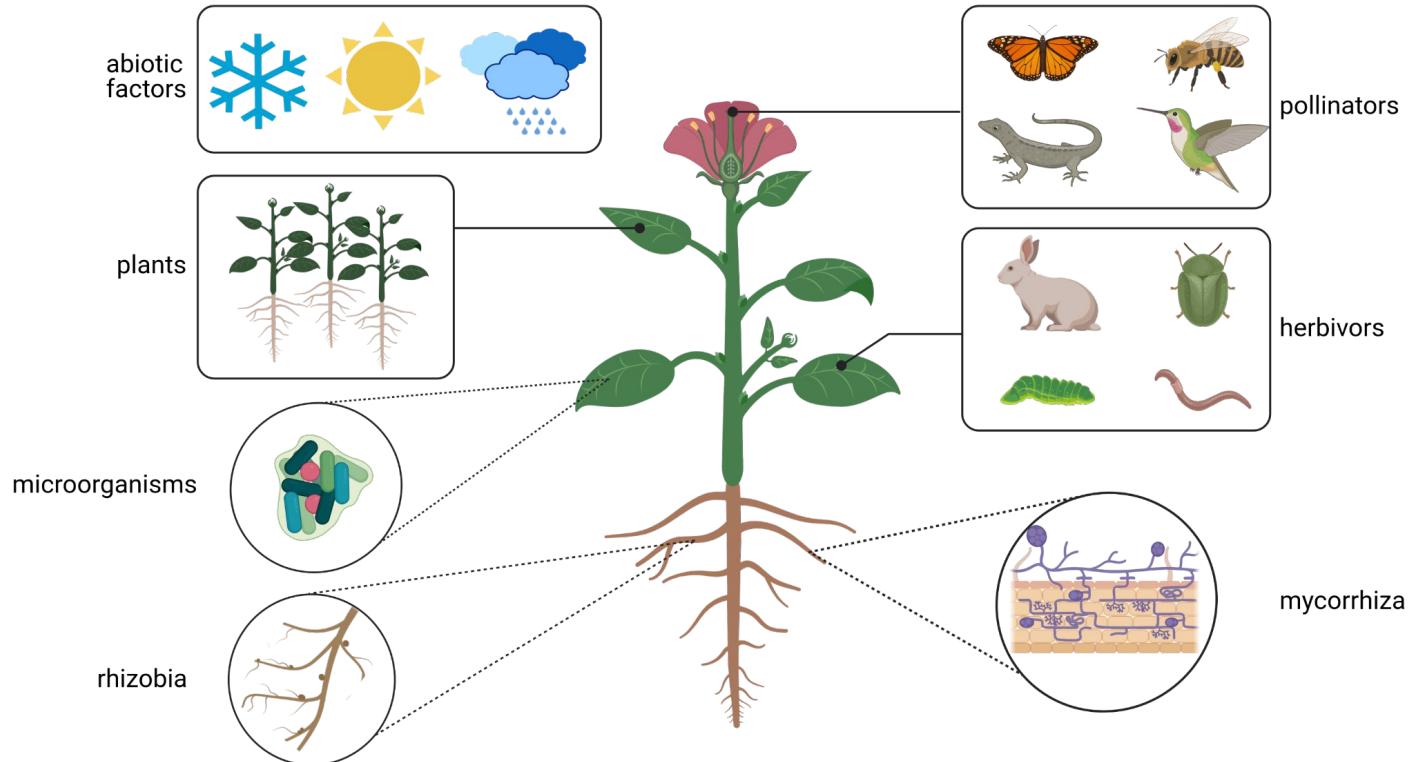
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- Translation
- Lipids: membrane vs. storage
- Carbohydrates: sugar, starch, cellulose
- Nitrate assimilation and metabolism

# What are specialized plant metabolites?

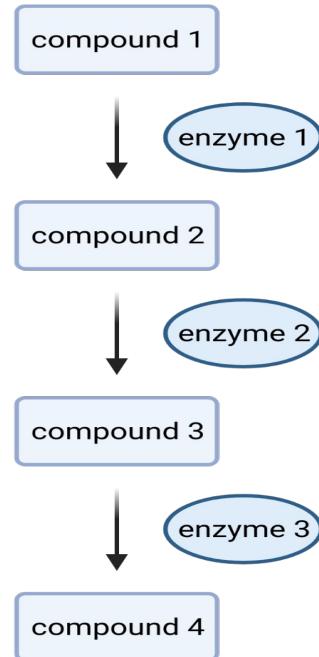
- Definition: metabolites conferring evolutionary advantage, but not immediately required for survival
- *Papaver somniferum*: morphine (pain)  
Mark Nesbit and Delwen Samuel
- *Digitalis purpurea*: digoxin (congestive heart failure)
- *Taxus brevifolia*: paclitaxel (cancer)  
Jason Hollinger

# Why do plants produce specialized metabolites?

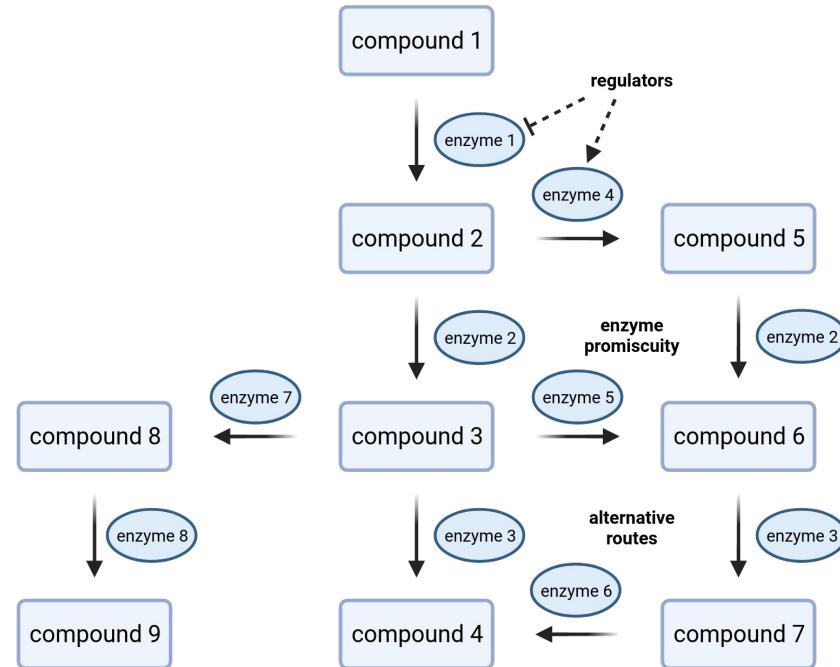


# How are specialized metabolites produced?

**Text book**

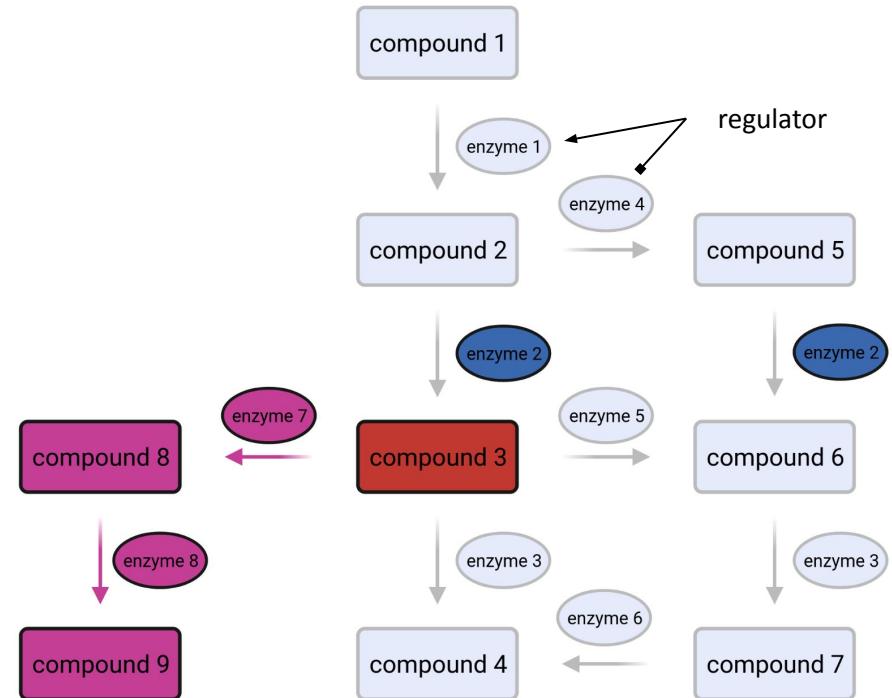


**Reality**



# 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



Anthocyanins

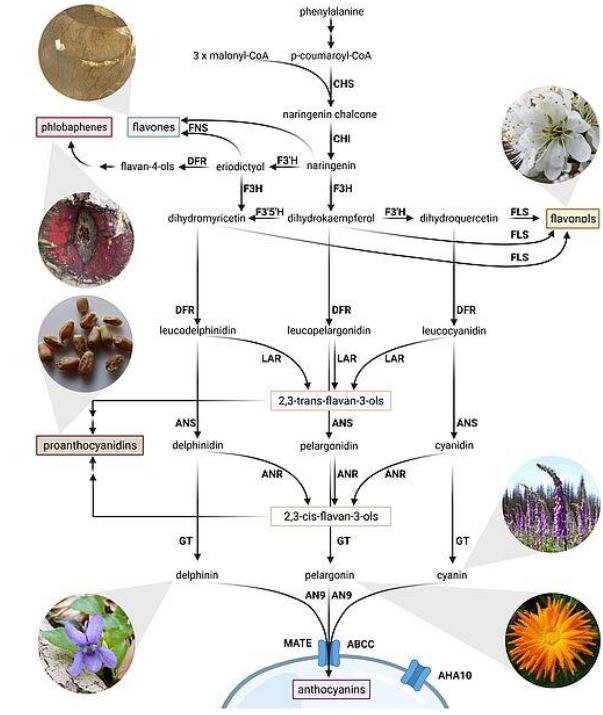
Carotenoids

Betalains



# Flavonoid biosynthesis branches

- Flavones
- Flavonols
- Proanthocyanidins
- Anthocyanins



- 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)



- 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

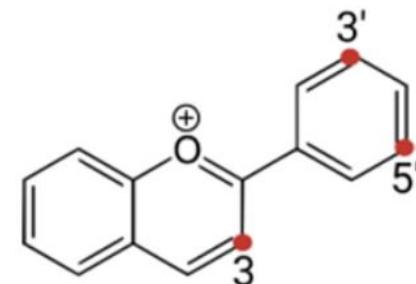
- Oligomeric or polymeric flavonoids composed of flavan-3-ol units (mainly catechin and epicatechin)
- Sources: grape seeds and skins, cocoa, apples, cranberries, blueberries, pine bark
- Biological roles: defense against herbivores and pathogens, contribute to astringency and color stability in fruits
- Chemical Features: colorless, often occur as polymers, stability influenced by pH, temperature, and degree of polymerization



# Anthocyanins - colorful pigments as model

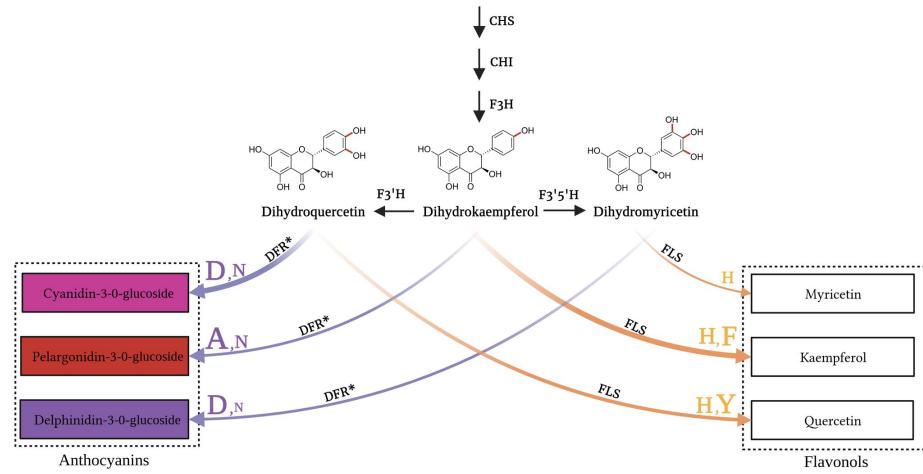


- Water-soluble flavonoid pigments responsible for the red, purple, and blue colors in many plants, fruits, and vegetables
- Glycosides of anthocyanidins — meaning an anthocyanidin molecule bound to one or more sugar molecules
- Based on the flavylium ion ( $C_{15}H_{11}O^+$ )
- Major anthocyanidins: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

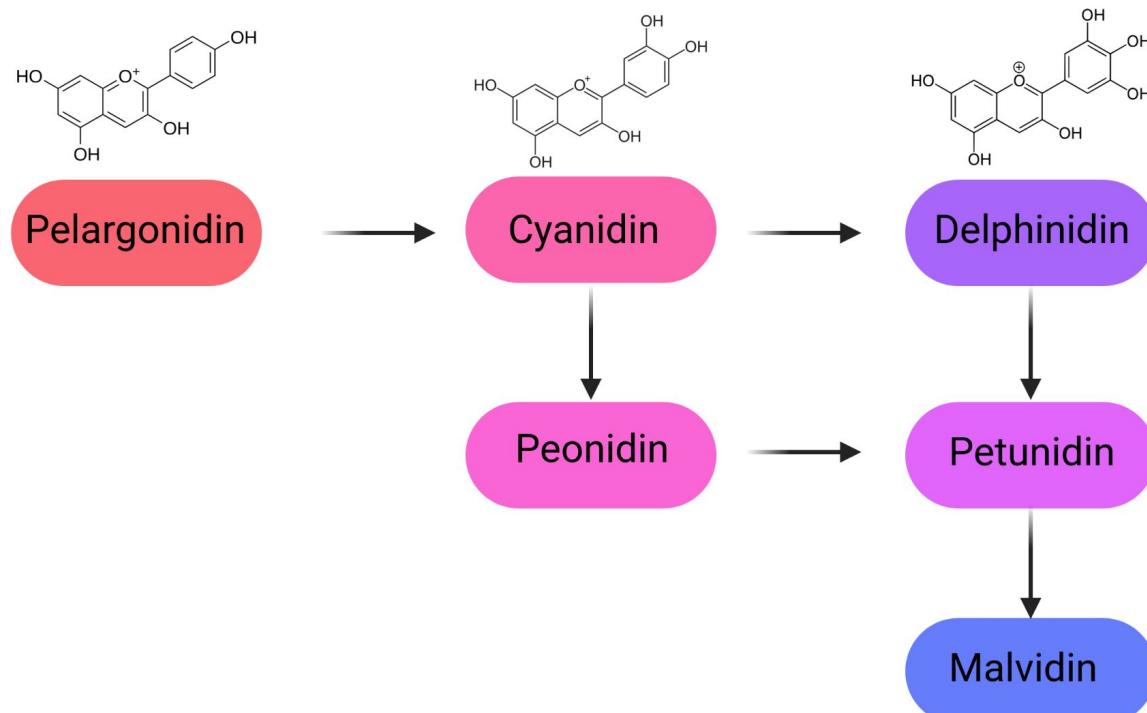


# Hydroxylation pattern

- 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

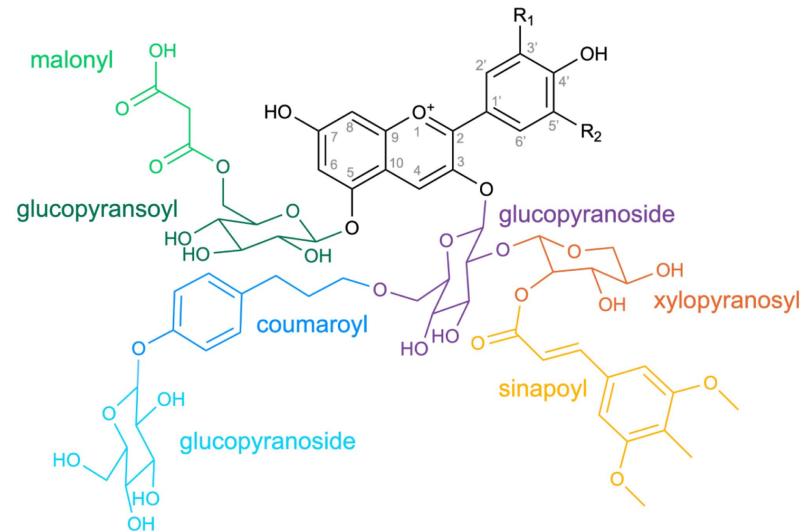


# Biochemical basis of color



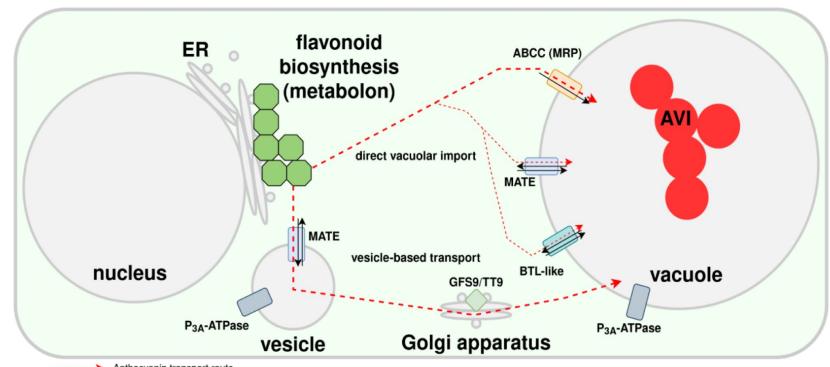
# Decoration of anthocyanins

- 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



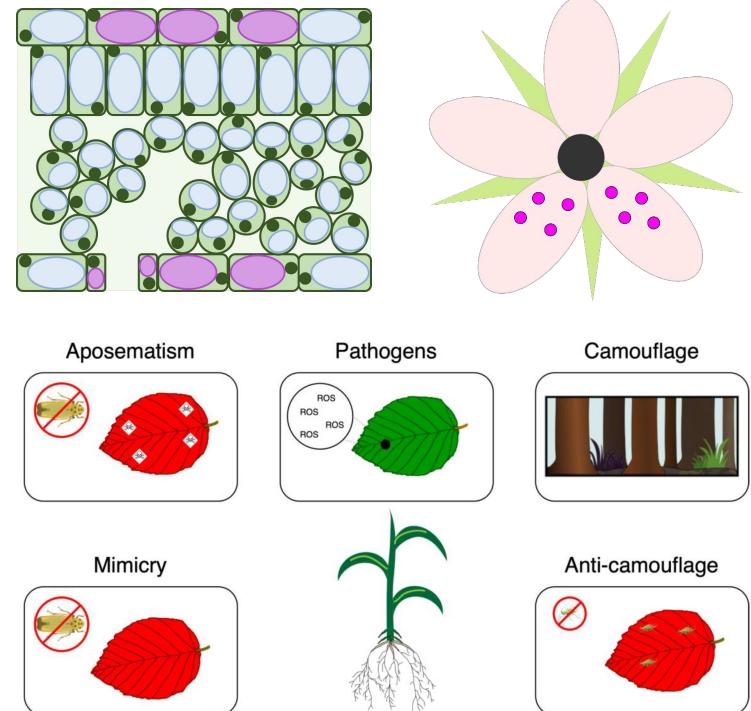
# Anthocyanin transport

- Anthocyanins accumulate in the centrale vacuole
- Postulated transport routes via vesicles or direct uptake across tonoplast
- Additional decoration in vacuole possible



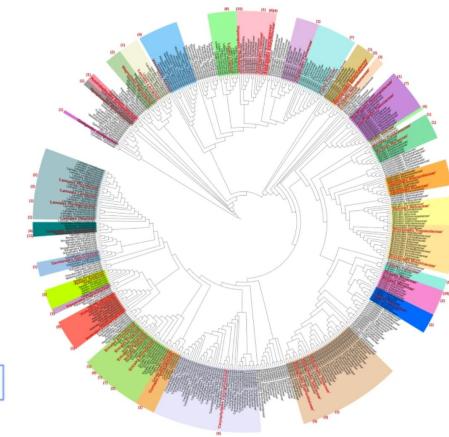
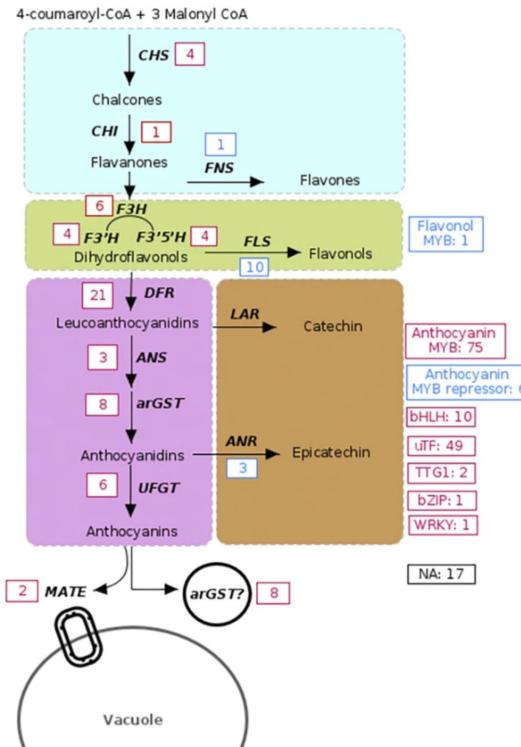
# Ecological functions of anthocyanins

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



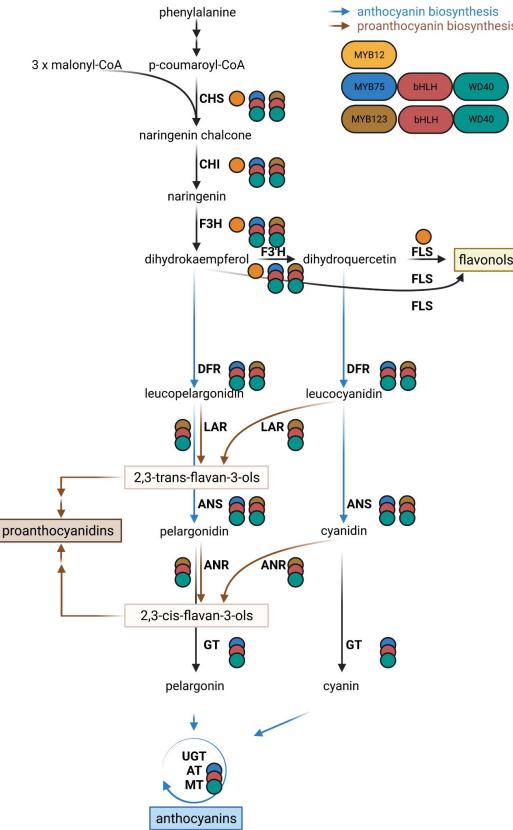
# Anthocyanins as a model system

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

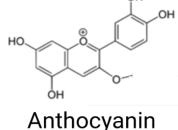
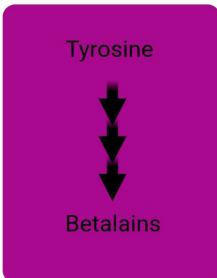
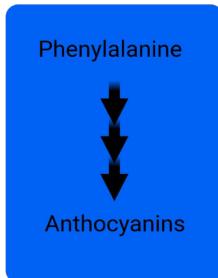


# Flavonoid biosynthesis mutants

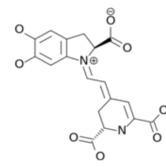
- Example 1: *fls* or *myb12*
  - Flavonols (-)
  - Anthocyanins (+)
  - Proanthocyanidins (+)
- Example 2: *chs*, *chi*, or *f3h*
  - Flavonols (-)
  - Anthocyanins (-)
  - Proanthocyanidins (-)
- Example 3: *bhlh*, *wd40*, *dfr*
  - Flavonols (+)
  - Anthocyanins (-)
  - Proanthocyanidins (-)



# Betalains



Anthocyanin color range

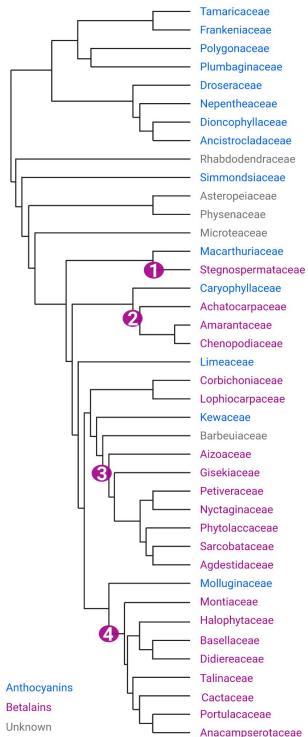


Betalain color range



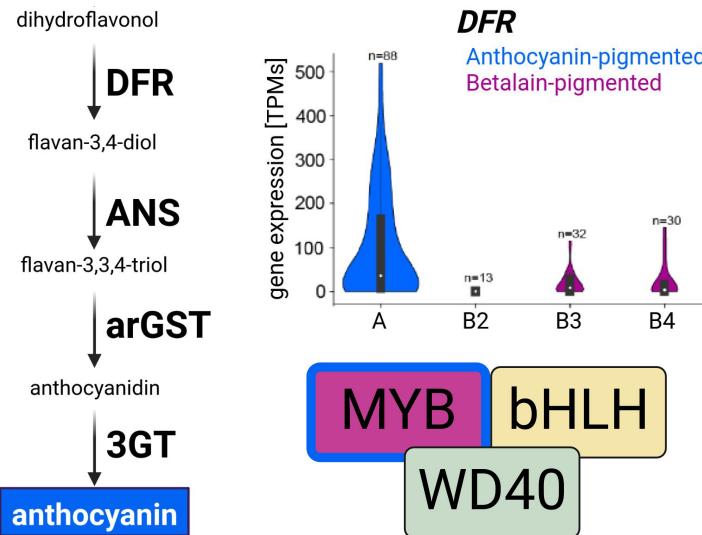
- Betalains are based on tyrosine
- Colors range from pink to yellow
- Same flower color can be caused by anthocyanins or betalains

# Complex pigmentation evolution in Caryophyllales

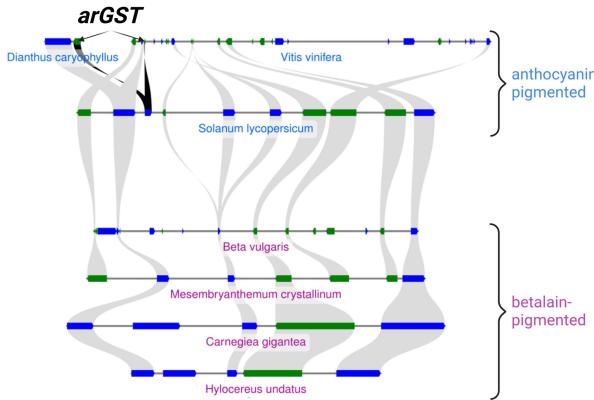


- Anthocyanin loss in entire plant families
- Anthocyanins and betalains appear functionally redundant
- Mutual exclusion: anthocyanins and betalains were never observed in same plants

# 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



- 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:  $(C_5H_8)^{\square}$

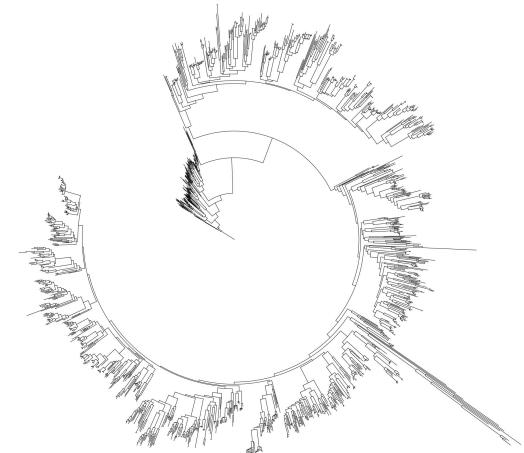
Type	No. of Isoprene Units	Carbon Atoms
Hemiterpenoids	1	$C_5$
Monoterpenoids	2	$C_{10}$
Sesquiterpenoids	3	$C_{15}$
Diterpenoids	4	$C_{20}$
Sesterterpenoids	5	$C_{25}$
Triterpenoids	6	$C_{30}$
Tetraterpenoids	8	$C_{40}$
Polyterpenoids	Many	Variable

- Convert prenyl diphosphate precursors into 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<sup>2+</sup> for substrate binding
- DDxxD and NSE/DTE motifs (bind metal ions and substrate diphosphates)
- RRX<sub>8</sub>W motif in many monoterpene synthases (involved in isomerization/cyclization)

Jeroen S. Dickschat  
(Organic Chemistry & Biochemistry)

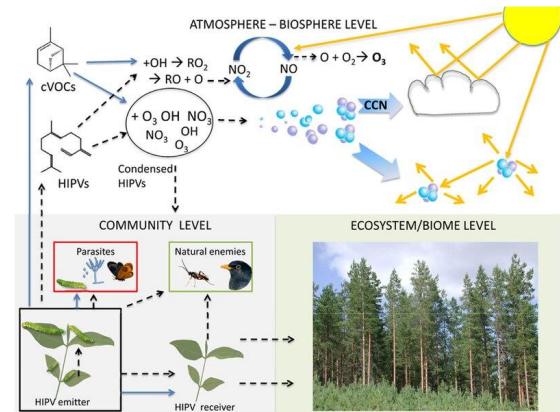
# Evolution of chemical diversity

- 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



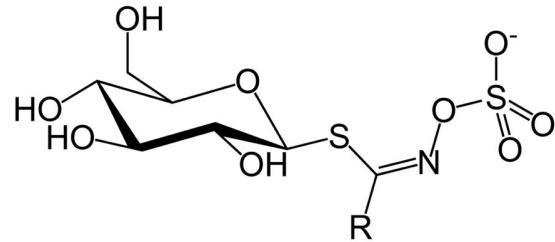
# Biological functions of terpenoids

- 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



# Glucosinolates

- Sulfur- and nitrogen-containing metabolites
  - $\beta$ -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: degradation to allyl isothiocyanate (mustard oil)
  - Glucoraphanin: degradation to sulforaphane (anticancer agent in broccoli)



## Glucosinolate ecology examples

- *A. thaliana*: insect attack leads to increase of aliphatic GSLs
- Diamondback moth (*Plutella xylostella*):
  - specialist herbivore on Brassicaceae
  - glucosinolate sulfatase (GSS) desulfates GSLs in its gut
  - desulfoglucosinolates not hydrolyzed by myrosinase (preventing mustard-oil)
- *Brassica juncea* (mustard):
  - levels of GSLs and myrosinase decline in seedlings as cotyledons mature
  - optimal defense theory: young, valuable tissues are better defended

- 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?