



HAL
open science

Plant cytochrome P450 plasticity and evolution

Cecilie Cetti Hansen, David R. Nelson, Birger Lindberg Møller, Daniele Werck Reichhart

► **To cite this version:**

Cecilie Cetti Hansen, David R. Nelson, Birger Lindberg Møller, Daniele Werck Reichhart. Plant cytochrome P450 plasticity and evolution. *Molecular Plant*, 2021, 14 (8), pp.1244-1265. 10.1016/j.molp.2021.06.028 . hal-03359133

HAL Id: hal-03359133

<https://hal.science/hal-03359133>

Submitted on 29 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Plant cytochrome P450 plasticity and evolution

Cecilie Cetti Hansen^{1,2,*}, David R. Nelson³, Birger Lindberg Møller^{1,2}
and Daniele Werck-Reichhart^{4,*}

¹Plant Biochemistry Laboratory, Department of Plant and Environmental Science, University of Copenhagen, Copenhagen, Denmark

²VILLUM Research Center for Plant Plasticity, University of Copenhagen, Copenhagen, Denmark

³Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN, USA

⁴Institute of Plant Molecular Biology, Centre National de la Recherche Scientifique (CNRS), University of Strasbourg, Strasbourg, France

*Correspondence: Cecilie Cetti Hansen (cich@plen.ku.dk), Daniele Werck-Reichhart (werck@unistra.fr)

<https://doi.org/10.1016/j.molp.2021.06.028>

ABSTRACT

The superfamily of cytochrome P450 (CYP) enzymes plays key roles in plant evolution and metabolic diversification. This review provides a status on the CYP landscape within green algae and land plants. The 11 conserved CYP clans known from vascular plants are all present in green algae and several green algae-specific clans are recognized. Clan 71, 72, and 85 remain the largest CYP clans and include many tax-specific CYP (sub)families reflecting emergence of lineage-specific pathways. Molecular features and dynamics of CYP plasticity and evolution are discussed and exemplified by selected biosynthetic pathways. High substrate promiscuity is commonly observed for CYPs from large families, favoring retention of gene duplicates and neofunctionalization, thus seeding acquisition of new functions. Elucidation of biosynthetic pathways producing metabolites with sporadic distribution across plant phylogeny reveals multiple examples of convergent evolution where CYPs have been independently recruited from the same or different CYP families, to adapt to similar environmental challenges or ecological niches. Sometimes only a single or a few mutations are required for functional interconversion. A compilation of functionally characterized plant CYPs is provided online through the Plant P450 Database (erda.dk/public/vgrid/PlantP450/).

Key words: land plants, green algae, diversification, promiscuity, neofunctionalization, convergent evolution, functionalities, plant metabolism

Hansen C.C., Nelson D.R., Møller B.L., and Werck-Reichhart D. (2021). Plant cytochrome P450 plasticity and evolution. *Mol. Plant.* **14**, 1244–1265.

INTRODUCTION

Cytochromes P450 (CYPs) constitute a superfamily of hemethiolate enzymes that catalyze an impressive arsenal of reactions. CYPs are ancient enzymes, present in all kingdoms of life, and they have particularly diversified in the plant kingdom. The high number and functional diversity of plant CYPs provide a molecular basis for plant plasticity as CYPs are involved in the formation of membrane sterols, phytohormones and signaling molecules, biopolymers for structural support and for protection from desiccation, UV protectants, pigments, volatile organic compounds for biotic and abiotic interactions, and a range of lineage-specific specialized metabolites with diverse roles, e.g., in pest and herbivore interactions.

The CYPomes (the total number of CYPs in a given species) of plants are usually much larger than those found in animals or microorganisms. The CYPs started to diversify early in plant evolution, with 39 CYP genes detected in the green algae *Chlamydomonas reinhardtii*, and 71 in the moss *Physcomitrium patens* (previously *Physcomitrella patens*; Nelson, 2006; Rensing et al.,

2020). In some flowering plants (angiosperms), CYPomes constitute up to 1% of the protein-coding genes (Nelson and Werck-Reichhart, 2011). This is exemplified by the 279 annotated full-length CYP genes in grape vine (*Vitis vinifera*) (Ilic et al., 2018), 329 in rice (*Oryza sativa*; Nelson and Werck-Reichhart, 2011), 443 in *Eucalyptus grandis*, and 741 in wheat (*Triticum aestivum*). Notably, when the total number of CYP sequences includes pseudogenes and short fragments, it increases significantly, reaching 579 and 798 in *V. vinifera* and *E. grandis*, respectively, illustrating the dynamic nature of CYPomes, with multiple gene duplications and losses.

CYPs are classified into families based on their amino acid sequence identities and phylogenetic clustering (Box 1). With the exponential number of CYP sequences becoming available from genomic and transcriptomic data without associated functional characterization, it is important that the global

Box 1. The CYP nomenclature system

In the cross-kingdom nomenclature system (Nelson, 2009), CYPs with more than 40% amino acid sequence identity are listed within the same family, and CYPs that share more than 55% amino acid sequence identity are grouped into the same subfamily. Plant CYPs are assigned to families with the numbers 51, 71–99, 701–999, and from 7001 onward. Subfamilies are designated by a letter after the number. CYP families are further separated into clans defined by the deepest distinct branching phylogenetic clades that are reproducible in multiple phylogenetic trees.

Family creep may stretch the 40% boundaries for family classification when a new sequence phylogenetically groups with an existing CYP family, but is slightly below the identity threshold (Nelson and Werck-Reichhart, 2011). Accordingly, on a few occasions, family merger has occurred where CYP83, CYP99, and CYP726 have been absorbed into the large CYP71 family, CYP723 has merged into the CYP89 family, and CYP705 is now viewed as a Brassicales-specific subfamily of CYP712. The old names are retained for historical reasons and to avoid confusion in the literature. On the contrary, family split has also occurred a couple of times, where addition of more sequences to the named collection resulted in a phylogenetic split. Consequently, CYP72B and CYP709A were renamed CYP734 and CYP735, respectively. Family split has also occurred for the CYP90B subfamily, which now groups with CYP724, but CYP90Bs have retained their original names to avoid confusion.

A new challenge for the nomenclature arises from adding evolutionarily distant CYPs. At more distant nodes, the CYP lineages converge to fewer ancestral sequences and this creates a naming problem. For example, two or more angiosperm CYP families may diverge from one gymnosperm family and each be >40% identical to that phylogenetic distinct gymnosperm family. Accordingly, the gymnosperm family must be given a new name and this breaks the 40% rule. The large fern-specific family CYP981 is an example where members of CYP981 are >40% identical to CYP92, CYP93, and CYP736 (Thodberg et al., 2021).

scientific community maintains and adheres to this nomenclature system to facilitate exchange of scientific knowledge. In 1990, CYP71A1 from avocado (*Persea americana*) was the first plant CYP to have its full sequence determined and named (Bozak et al., 1990). In 2018, almost 30 years later, the number of named CYPs in the plant kingdom passed 16 000 (Nelson, 2018). This number has kept steeply increasing, with over 32 000 plant CYPs distributed over 670 families named to date. Some CYP families are ubiquitous among kingdoms (CYP51) or land plants (e.g., CYP73 and CYP78), whereas others are lineage-specific (e.g., CYP702 and CYP708 in Brassicaceae).

The large number of plant CYP families reflects a diverse amino acid sequence space. BLAST alignments down to the low 20% identity range are observed between plant CYPs. Despite this, all CYP proteins share a common three-dimensional (3D) architecture dominated by the presence of α helices and a smaller proportion of β sheets (Lee et al., 2008; Li et al., 2008; Fujiyama et al., 2019; Gu et al., 2019; Niu et al., 2020; Zhang et al., 2020). A unique feature is the cysteine-linked covalently bound heme group in the catalytic center. Initially described for mammalian CYP2, six substrate recognition sites (SRSs; Gotoh, 1992) that frame the distal heme region are key determinants of catalytic specificity. Substrate-access channels may also contribute to the specificity of a CYP by controlling access of substrates to the active site. Microsomal CYPs are membrane-bound through an N-terminal membrane helix that restricts its mobility and provides an anchor point focusing the interaction with up- and downstream pathway partners, including the obligatory electron donor protein cytochrome P450 oxidoreductase (POR; Knudsen et al., 2018). Isoforms of cytochrome b_5 may also serve as electron donors.

The plant CYPome is highly dynamic in evolutionary terms and the families, subfamilies, as well as the proportion of CYP genes belonging to each of them, can vary greatly between taxonomic groups. These differences translate into qualitative and quantitative differences in metabolite profiles, sometimes visually by morphological or developmental phenotypes, and by adaptation to specific ecological niches. Lineage-specific gene proliferation of paralogs is often termed “blooms” (Feyereisen, 2011). A CYP

family bloom is, for example, found in *O. sativa* with 90 full-length CYP71s (88 CYP71 plus two CYP99 subfamily genes), some members of which are reported to be involved in diterpenoid phytoalexin biosynthesis (e.g., Wang et al., 2011; Wu et al., 2011; Zhan et al., 2020). Another example is the fern *Cystopteris utahensis*, where 44 different CYP981 transcripts were identified from 1kP transcriptomes (One Thousand Plant Transcriptomes, 2019), although none of them has been assigned a function.

The number of functionally characterized CYPs is steadily increasing, and more than 800 plant CYPs have been characterized by their ability to metabolize one or more substrates. These are collected in The Plant Cytochrome P450 Database (erda.dk/public/vgrid/PlantP450/), which provides an extensive compilation of CYPs known to metabolize one or more substrates and includes references for further exploration. The classic CYP-catalyzed reaction is a monooxygenation of a carbon atom. The CYP-mediated molecular oxygen activation can lead to the formation of alcohols, ketones, aldehydes, carboxylic acids, and epoxides, but other types of reactions, such as oxidative rearrangement of carbon skeletons (e.g., Nasomjai et al., 2009), oxidative C–C bond cleavage (e.g., Irmier et al., 2000), dehydration (e.g., Hansen et al., 2018), dealkylation (e.g., Bak et al., 1997), desaturation (e.g., Morikawa et al., 2006a), and decarboxylation (e.g., Koch et al., 1995) can also result from radicals or reactive oxygenated intermediates generated by CYPs. Formation of methylenedioxy bridges (e.g., Ikezawa et al., 2003), lactone rings (e.g., Kim et al., 2005), and rings harboring several heteroatoms (Klein and Sattely 2015), oxidative C–C and C–O phenol coupling reactions (e.g., Ikezawa et al., 2008; El-Awaad et al., 2016), ring expansion (e.g., Nett et al., 2020), ring contraction (e.g., Helliwell et al., 2001), formation of allene oxides (e.g., Howe et al., 2000), N-oxygenations (e.g., Koch et al., 1995; Sibbesen et al., 1995), isomerization reactions as observed in oxime metabolism (Halkier et al., 1989; Clausen et al., 2015), and aryl migration reactions as observed in isoflavonoid biosynthesis (Akashi et al., 1999) are other outcomes of CYP activity. We refer to other reviews for a detailed description of catalytic mechanisms

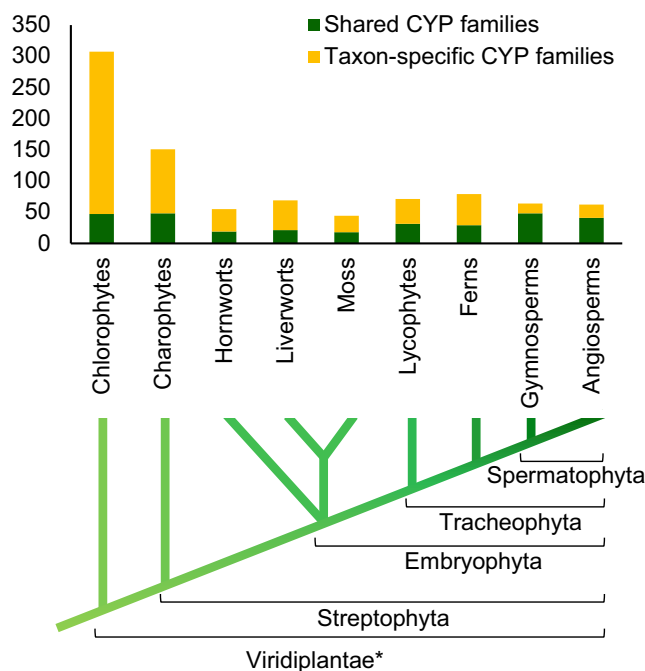


Figure 1. Abundance of CYP families through plant evolution.

The total number of known CYP families is divided into families that are shared between two or several taxonomic groups and CYP families that are specific to a given taxon. The estimated numbers of species in the nine taxa are: chlorophytes ~13 000; charophytes ~9000; hornworts 200–250; liverworts ~7500; mosses ~12 000; lycophytes ~1300; ferns ~10 500; gymnosperms ~1000; angiosperms ~300 000 (Cox et al., 2010; Villarreal et al., 2010; Von Konrat et al., 2010; Guiry, 2012; Christenhusz and Byng, 2016; PPG I, 2016). The number of CYP families found in land plants has shown signs of saturation, whereas more CYP families from green algae are expected to be identified. *The newly defined phylum within Viridiplantae, the Prasinodermophyta (Li et al., 2020), is not included in the figure.

of unusual plant CYP reactions (Mizutani and Sato 2011; Zhang and Li 2017). Generally, the mechanism of CYP attack is the same, and most of the unusual CYP reactions only diverge in a subsequent rearrangement of a reaction intermediate or a labile product (Guengerich and Munro 2013).

The diverse sets of reactions catalyzed by CYPs all contribute to expansion of the chemical space and decoration of carbon backbones with oxygen providing activated sites enabling further expansion of the chemical space by, for example, O-methylation, O-glycosylation and O-acylation. In this review, we provide an update on plant CYP families within green algae and land plants, and discuss molecular features and dynamics of CYP plasticity and evolution, highlighted along with specific examples from various biosynthetic pathways representing intriguing evolutionary events.

CYP FAMILIES THROUGH PLANT EVOLUTION

The plant lineage Viridiplantae covers green algae, land plants, and a recently defined new phylum of marine algae, the Prasinodermophyta, established to be the earliest diverging phylum within Viridi-

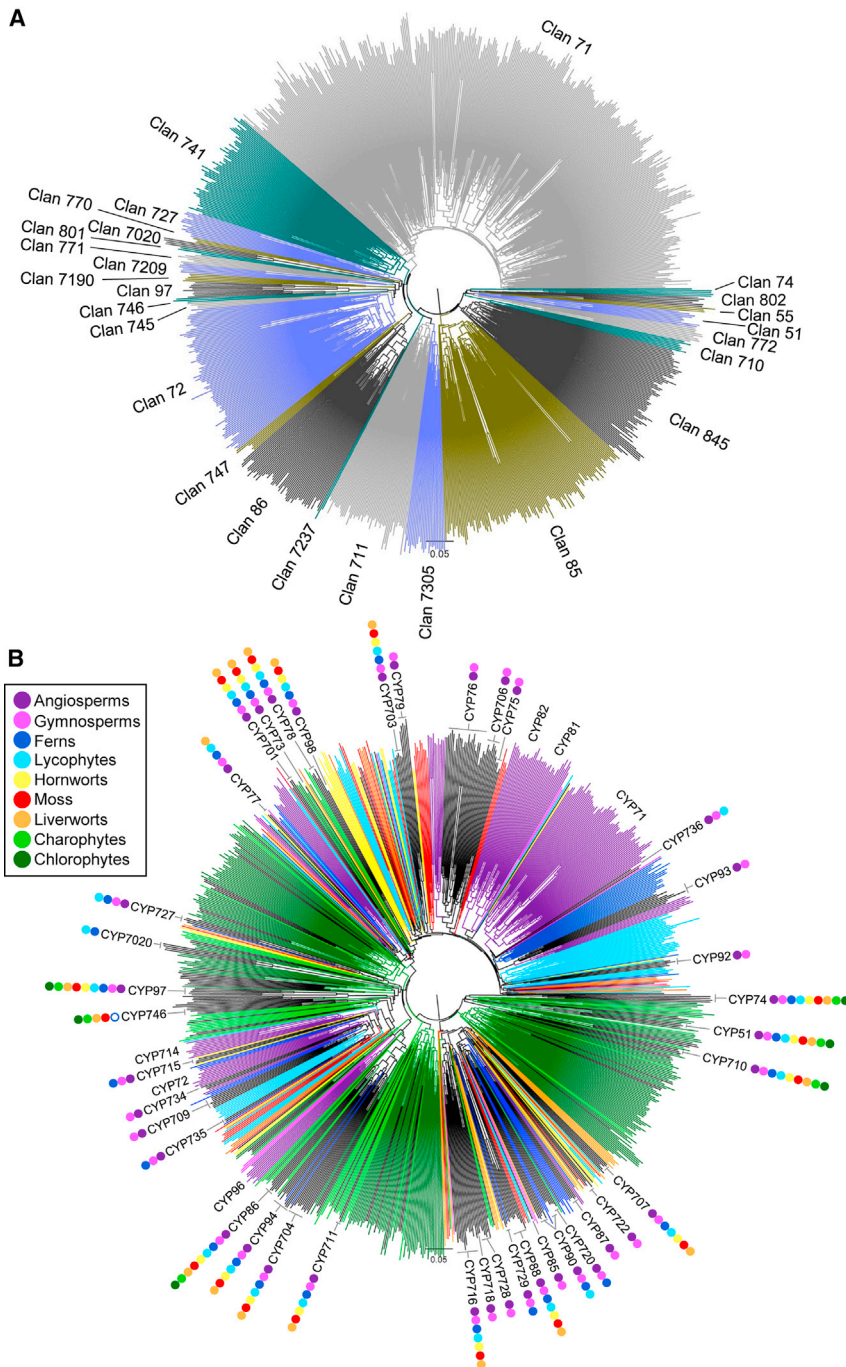
plantae (Li et al., 2020). Chlorophyte and charophyte algae are collectively known as green algae. Chlorophytes comprise 12 known algal classes, while charophytes comprise a paraphyletic assemblage of five algal classes in the division Streptophyta (charophyte algae and land plants) (Guiry, 2012; Becker, 2013). The origin of land plants (embryophytes) may be traced back to terrestrial charophyte algae (Delwiche and Cooper, 2015; Harholt et al., 2016). Changes in biochemistry, accompanied with innovations in anatomy and signaling, have been a prerequisite for successful evolution of land plants (Rensing, 2020). Appearance of some CYP families during plant evolution is directly linked to the occurrence of specific metabolite classes, such as carotenoids, phytohormones, oxygenated lipids, or phenylpropanoids. Given their prevalent occurrences in diverse plant metabolic pathways, CYPs can be used to infer the development of metabolic systems throughout plant evolution (Nelson and Werck-Reichhart, 2011; Cannell et al., 2020). This section provides a status on occurrence of plant CYP families from chlorophyte algae to angiosperms, as an update to the 2011 review by Nelson and Werck-Reichhart. Catalytic functions of conserved CYP families dating back to green algae and CYP families with recently elucidated functions are emphasized.

Occurrence of CYP families

Many green algae CYP sequences and CYP genes from the newly sequenced unicellular marine algae *Prasinoderma coloniale* have been identified and named within the past decade. The *P. coloniale* genome (Li et al., 2020) contains 13 CYP genes in nine families (CYP51, CYP97, CYP710, CYP745, CYP746, CYP800, CYP804, CYP7196, and CYP7200). These may represent a sample of CYPs from the earliest green plants before chlorophytes and charophytes diverged. Chlorophyte algae have 1199 CYP sequences named from 117 species distributed in 275 CYP families, and charophyte algae have 1320 sequences named from 62 species distributed in 115 CYP families (supplemental data 1 and 2). A resource of named CYPs from green algae is provided in supplemental data 2. Algal CYPomes are smaller than those of vascular plants (tracheophytes) and represent up to ~0.2% of protein-coding genes in some algal species. The chlorophyte *Volvox carterii* has 19 CYP genes, *Trentepohlia annulata* has 58, and most chlorophytes fall in between these sizes, like *C. reinhardtii* with 39 CYP genes. In the partially annotated genome of the charophyte *Penium margaritaceum*, 46 CYP genes have been recorded so far.

In green algae, CYPs are highly diversified. Green algae is the plant group featuring the highest number of CYP families, the majority of which are algae specific (Figure 1, supplemental data 3). To date, the number of algal CYP families has not shown signs of saturation and there are probably many more to be discovered. Only 41 CYP families are shared between chlorophyte and charophyte algae (about 12%), showing the significant divide between the two groups. Fungal CYP55 (nitric oxide synthase) is also present in chlorophytes, but not in charophytes, which, together with high sequence identity, might indicate lateral transfer of CYP55 after the two algal clades split.

Figure 2 shows the phylogenetic distribution of CYP families in green algae and land plants. CYP clans 51, 74, 97, 710, 711, and 746 were previously known as single-family clans (Nelson and



Werck-Reichhart, 2011), but annotation of more algal sequences has changed this picture, revealing three green-algae-specific CYP families in the 51 clan, four in the 710 clan, no less than 46 in the 711 clan, and possibly two in the 746 clan (supplemental data 1). These clans are still represented by a single CYP family in land plants, and clans 74 and 97 remain single-family clans within Viridiplantae. Remarkably, classification of the many green algal sequences shows that all CYP clans known from land plants are present in green algae. In addition to the conserved clans, green algae possesses 14 algae-specific clans.

Families common to chlorophytes and charophytes that are conserved throughout land plants are CYP51, CYP74, CYP97,

Figure 2. Phylogeny of CYP families within green algae and land plants.

(A) Branches are colored according to clan distribution.

(B) Branches are colored according to taxonomic group: dark green, chlorophytes; light green, charophytes; orange, liverworts; red, moss; yellow, hornworts; light blue, lycophytes; dark blue, ferns; pink, gymnosperms; purple, angiosperms. Black branches represent CYP families that are shared across two or more taxonomic groups. Some of the conserved CYP families are labeled with colored dots according to their presence in certain taxa. The blue open circle for CYP746 illustrates that this family has only been found in very few ferns. Colored branches show taxon-specific CYP families. Most CYP families are represented by one sequence per family but some families are represented by more than one sequence to help stabilize their position on the tree. Amino acid sequences were collected from the latest updated version of the Cytochrome P450 Homepage (Nelson, 2009) and 105 of the green algae sequences were obtained from Cooper and Delwiche (2016). Amino acid sequences (954) were aligned using Clustal Omega and phylogeny was inferred using the neighbor-joining method. A high-resolution phylogenetic tree with tip labels is available in supplemental Figure 1.

and CYP710. A couple of CYP families appeared in green algae and are conserved in some land plants but were lost during evolution (supplemental data 1). For example, the CYP746 family (clan 746) is present in algae, moss, liverworts, and a few ferns, but not in hornworts or seed plants (spermatophytes). This is the only example of a CYP clan that is present in early-diverging plant lineages and lost upon evolution of vascular plants. The CYP765 family (clan 72) is conserved from green algae to gymnosperms and lost in angiosperms.

Mining of the 1kP transcriptomes and genomes of early land plants revealed many taxon-specific families (Figure 1; supplemental data 1 and 2), some of which show great abundance, such as the fern-specific CYP981 family (clan 71), which is the richest CYP family in this taxon (Thodberg et al., 2021). Other CYP families are shared between a few taxa (supplemental data 1). For example, family CYP825 (clan 71) is found in liverworts and hornworts, and families CYP7020 (clan 7020), CYP7332, and CYP7333 (both clan 71) are found in ferns and lycophytes. The new 7020 clan is present only in ferns and lycophytes. Other CYP families show yet a broader distribution, but are still limited to a few neighboring taxa. This applies to CYP947 family members (clan 85) that are spotted only in ferns, lycophytes, and gymnosperms. A list of named CYPs, including protein sequences from bryophytes, lycophytes, ferns, and gymnosperms, is available in supplemental data 2.

Functionalities of conserved CYP families

The CYP families CYP51, CYP710, CYP97, CYP74, and CYP86 are conserved from green algae and throughout land plants, carrying out essential catalytic reactions in metabolism, and five families (CYP51, CYP710, CYP97, CYP74, and CYP704) are present across several kingdoms. CYP704 is, however, not found in green algae. The families tend to be maintained as a single copy or at low copy numbers, although retention of duplicates and recruitment to a specialized pathway also occur.

CYP51G functions as an obtusifoliol 14 α -demethylase in the biosynthesis of membrane sterols (e.g., Bak et al., 1997). This essential CYP51 function is conserved in other kingdoms, like fungi and animals, but with enzymes using lanosterol as substrate. Grasses (family Poaceae) have evolved a new subfamily, CYP51H, with eight genes in *O. sativa* and six in oat (*Avena strigosa*). The CYP51H10 gene (also known as SAD2) from *A. strigosa* has been characterized. It catalyzes epoxidation and hydroxylation of β -amyirin in the formation of the toxin avenacin (Qi et al., 2006; Kunii et al., 2012; Geisler et al., 2013).

CYP710A is a sterol C-22 desaturase, also contributing to the formation of membrane sterols in land plants (Morikawa et al., 2006a). Occurrence of sterols containing a double bond at C-22 in the sterol side chain is a characteristic of land plant and fungi sterol profiles (Morikawa et al., 2006b), and CYP710 shares a common ancestor with its functional homologs in fungi (CYP61). Subfamily CYP710A is specific to Streptophyta, while subfamily CYP710B is present in green algae. CYP710 genes are also found in other algae and in some protists, documenting that the family is very ancient.

The CYP97 family includes key enzymes in the biosynthesis of carotenoid pigments, which have vital roles in photosynthesis and photoprotection. CYP97 genes are not restricted to the plant kingdom and have also been identified in several protists. Some protists have plastids acquired by tertiary symbiosis of green algae (Keeling, 2010), and they may have obtained CYP97 genes by such symbiosis. Plant CYP97s catalyze ring hydroxylation of α - and β -carotenes and their monohydroxylated derivatives, a function that is conserved from green algae and through land plants (Kim et al., 2009; Liang et al., 2020). Neofunctionalization of a CYP97 for production of the acyclic diterpene plaunotol, known for its anti-gastric-ulcer properties, has been reported for *Croton stellatopilosus*. Here, CsCYP97C27 catalyzes C18-hydroxylation of geranylgeraniol to form plaunotol (Sintupachee et al., 2014).

The plant CYP74 family has previously been dated back to liverworts (Nelson and Werck-Reichhart, 2011), but investigation of more algae and early land plant datasets shows presence of CYP74 members in hornworts, charophytes, and freshwater chlorophytes. CYP74 genes have also been identified in some animal species, such as cnidarians. CYP74s may thus predate the split between animals and plants or the cnidarian copies must be a result of lateral transfer. The rarity of CYP74 outside the plants favors a lateral transfer otherwise many gene losses would be required. Members of the plant CYP74 family are involved in the metabolism of oxylipins, including jasmonic acid, which functions in cell signaling and defense (Wasternack

and Feussner, 2018; Farmer and Goossens, 2019). They are atypical CYPs, not requiring electron donation and molecular oxygen, and use fatty acid hydroperoxides as substrates. Land plants typically contain a few CYP74 genes from different subfamilies. CYP74As usually function as allene oxide synthases (AOSs) to form 12-oxo-phytodienoic acid, a precursor of the essential plant signaling compounds jasmonic acid and derivatives. Other CYP74s also metabolize fatty acid hydroperoxides, but act as hydroperoxide lyases (HPLs), divinyl ether synthases (DESSs), or epoxyalcohol synthases (EASs). The charophyte *Klebsormidium flaccidum* contains a single CYP74 (CYP74Z1), which functions as an AOS (Koeduka et al., 2015). The presence of a single CYP74 has also been observed in the charophytes *Klebsormidium subtile*, *Coleochaete scutata*, and *Entransia fimbriata*. In addition to angiosperms, AOS, HPL, and DES activities have been identified in bryophytes and lycophytes (Scholz et al., 2012; Koeduka et al., 2015; Gorina et al., 2016). These activities are scattered on various CYP74 subfamilies, suggesting that the activities have arisen multiple times in plant evolution. Along this line, a single or few mutations have been shown sufficient to partly or fully change AOS to HPL activity (Lee et al., 2008; Toporkova et al., 2008), DES to AOS activity (Toporkova et al., 2013), or AOS to EAS activity (Toporkova et al., 2020), showing that only a few amino acid changes are needed for a diverse set of functional interconversions.

Presence of CYP86 members across the plant lineage has also been broadened and now dates back to chlorophytes. Notably, this family has more subfamilies than the other conserved CYP families (supplemental data 1). CYP86s catalyze oxygenation of fatty acids at the omega position (Bak et al., 2011), forming hydroxylated fatty acids, but also more oxidized derivatives such as dicarboxylic acids. Thereby, they generate a broad range of precursors for formation of waterproof biopolymers like cutin and suberin, essential for water transport or protection from water loss. CYP86 in green algae were thus most likely required to protect spores from desiccation, and they represented a critical asset for plant terrestrialization. Besides, CYP86s have also been reported to positively regulate the plant immune system, with high impact on defense against pathogens both in *A. thaliana* and sea island cotton (*Gossypium barbadense*) (Xiao et al., 2004; Wang et al., 2020a). CYP704, which is ancestral to CYP86 and is also described as a fatty acid ω -hydroxylase in angiosperms (Dobritsa et al., 2009), is present in land plants, protists, and red algae but was lost in green algal lineages.

CYP families with recently elucidated functionalities

Despite numerous CYPs being characterized in recent years, several CYP families are still without known function. On the positive side, functions have been assigned to some members of CYP families that were listed as orphan in the 2011 review. These include family CYP716 from the clan 85 and families CYP712, CYP736, and CYP750 from the 71 clan. In addition, the role of CYP711s (711 clan) and CYP722s (clan 85) in strigolactone biosynthesis is now better understood.

Many eudicot CYP716s have been shown to metabolize triterpenes. This family possibly evolved from a duplication of genes

involved in brassinosteroid metabolism, resulting in activities contributing to biosynthesis of triterpenoids with a defense function in land plants. Several CYP716As catalyze a three-step C28 oxidation of β -amyrin, α -amyrin, and/or lupeol, but oxygenation of various carbon atoms in the triterpene backbone has also been reported (e.g., Fukushima et al., 2011; Miettinen et al., 2017). A few CYP712Ks were found to oxygenate the triterpenoid friedelin at C29, resulting in formation of polpunoic acid in the biosynthesis of celastrol (Bicalho et al., 2019; Hansen et al., 2020). Some members of the CYP736 family oxygenate small, often volatile compounds: CYP736A2 from *Lotus japonicus* converts valine- and isoleucine-derived oximes to their corresponding α -hydroxynitriles in cyanogenic glucoside biosynthesis (Takos et al., 2011), CYP736A107 from *Sorbus aucuparia* and CYP736A163 from *Malus \times domestica* are biphenyl 4-hydroxylases (Sircar et al., 2015), and CYP736A167 from *Santalum album* hydroxylates sesquiterpenes like santalene and bergamotene (Celedon et al., 2016). CYP736A304 and CYP736A305 from *Nicotiana attenuata* produce 17-hydroxygeranylinalool for defense against herbivores (Li et al., 2021). CYP750B1 catalyzes hydroxylation of the monoterpene sabinene in thujone biosynthesis (Gesell et al., 2015). CYP749A1 has been linked to the arising tolerance to herbicides in upland cotton (*Gossypium hirsutum*), but its activity in plant metabolism is still unknown (Thyssen et al., 2018).

Strigolactones are involved in the symbiotic relation between land plants and soil arbuscular mycorrhiza and function as endogenous plant hormones (Moreno et al., 2021). In land plants, CYP711As (also known as MAX1) oxygenate the intermediate carlactone in strigolactone biosynthesis (Abe et al., 2014; Zhang et al., 2014, 2018; Yoneyama et al., 2018; Mori et al., 2020). Most of the functionally characterized CYP711As convert carlactone to carlactonoic acid, but *Selaginella moellendorffii* CYP711A17v1 and CYP711A17v3 and *O. sativa* CYP711A2 further catalyze the BC-ring closure proceeding via 18-hydroxy carlactonoic acid and resulting in 4-deoxyorobanchol. In addition, hydroxylation of 4-deoxyorobanchol to orobanchol has been shown for Os-CYP711A3 (Zhang et al., 2014; Yoneyama et al., 2018). CYP722Cs act downstream of the most common CYP711A reaction and convert carlactonoic acid to orobanchol, or to the stereoisomer 5-doxystrigol in some plants such as tree cotton (*Gossypium arboreum*) (Wakabayashi et al., 2019, 2020).

In some other cases, such as for CYP715 or CYP720A, activities in hormonal pathways have been suggested by observed physiological roles in flower development as revealed by null mutants, but no substrate was assigned (Liu et al., 2015; Chaturvedi et al., 2020). Some conifer-specific CYP720Bs, however, oxygenate several diterpene backbones (Ro et al., 2005; Hamberger et al., 2011; Geisler et al., 2016), which might constitute a lead to the family activity in hormone metabolism. The functions of CYP78s that were already known to control organ growth have been extended to cell fate maintenance and control of defense against a broad spectrum of pathogens, still without identification of the signal generated (Maeda et al., 2019; Poretska et al., 2020).

Only very few CYPs from taxa-specific CYP families in early land plants have been functionally characterized. These include

CYP788A1 from the lycophyte *S. moellendorffii* (Weng et al., 2010), CYP964A1 and CYP970A14 from the moss *Calohyphnum plumiforme* (Mao et al., 2020), and CYP981F5 from the fern *Phlebodium aureum* (Thodberg et al., 2021). Notably, these CYPs enable the species to produce compounds already known from angiosperms through similar or alternative metabolic routes (discussed further below).

EVOLUTION OF THE PHENYLPROPANOID PATHWAY MIRRORS LAND PLANT EVOLUTION

The CYP73, CYP98, and CYP84 families involved in the phenylpropanoid metabolic network eminently reflect some important evolutionary events in plant metabolism, both in terms of functions in the biosynthesis of key metabolites important for terrestrialization of plants and as illustrative examples of lineage-specific gene duplication and functional specialization.

Phenylpropanoids play key roles in the evolution of terrestrial plants, functioning as building blocks for structural biopolymers such as lignin, suberin, cutin, and sporopollenin, ensuring mechanical support, water retention and transport, but also serving as UV protectants, antioxidants, and defense compounds. Members of the CYP73/CYP98 clade are present in charophytes, possibly representative of their common ancestor (Renault et al., 2017b), but both CYP73 and CYP98 simultaneously emerged ~450 mya when bryophytes colonized land. They show very different duplication and diversification modes along land plant evolution.

CYP73

The second step in the core phenylpropanoid pathway is catalyzed by CYP73 (C4H) and is a *para*-hydroxylation of cinnamic acid (Figure 3). The CYP73 family is a low-copy-number family under high purifying selection with usually one to five CYP73 genes detected in plant genomes. In seed plants, a conserved gene duplication has occurred prior to the split between gymnosperms and angiosperms giving rise to an additional CYP73 (also called class II CYP73) (Renault et al., 2017b). While retained in many species, it was lost in most gymnosperms and in Brassicaceae. A second fixed gene duplication occurred in monocots. Functional analysis of the three CYP73 members from *Brachypodium distachyon* demonstrated highly efficient cinnamic acid hydroxylase activity for all of them. The class I and II enzymes, however, differ in the length of the N-terminal membrane anchor, where the class II CYP73 has an elongated anchor causing altered membrane topology. The exact physiological consequences of this feature are still unknown, but it might affect enzyme mobility and interaction with redox or pathway partners. Current data thus indicate that fixation of gene duplicates does not necessarily depend on altered catalytic activities but may result from other functional aspects leading to fitness increase. This argues that CYP localization, topology, but also expression pattern analyses in the cause of plant ontogeny need to be investigated to guide proper assessments of their physiological functions.

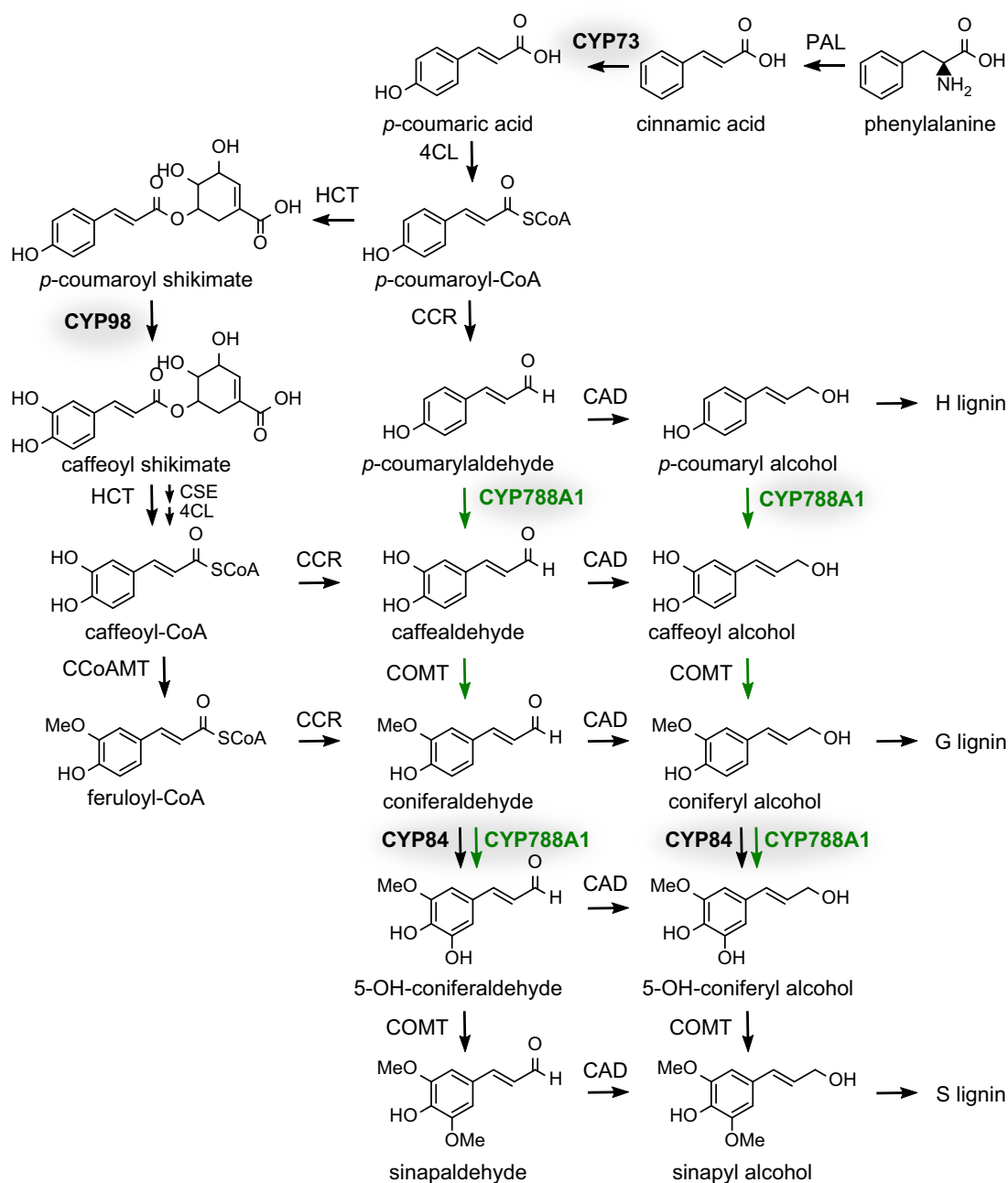


Figure 3. The phenylpropanoid metabolic network underlying the biosynthesis of H, G, and S-lignin monomers.

*Sm*CYP788A1 reactions are labeled in green and green arrows indicate the pathway specific to *Selaginella*. PAL, phenylalanine ammonia-lyase; 4CL, 4-coumarate-CoA ligase; HCT, hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase; CSE, caffeoyl shikimate esterase; CCoAMT, caffeoyl-CoA *O*-methyltransferase; COMT, caffeic acid *O*-methyltransferase; CCR, cinnamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase.

CYP98

At the entry of the lignin branch of the phenylpropanoid network, CYP98 members catalyze 3'-hydroxylation of *p*-coumaroyl-shikimate (Figure 3). They also efficiently catalyze hydroxylation of *p*-coumaroyl-quinic acid for the formation of chlorogenic acid. *Coffea canephora*, which accumulates chlorogenic acid as a major soluble phenolic, has two CYP98s. One of them (*Cc*CYP98A36) shows a clear preference for *p*-coumaroyl-shikimate, and the other (*Cc*CYP98A35) has specialized toward production of

chlorogenic acid while maintaining activity with *p*-coumaroyl-shikimate (Mahesh et al., 2007).

Despite its essential role in lignin biosynthesis in vascular plants, the appearance of CYP98 predates the occurrence of the lignin biopolymer. Genome mining for CYP98 members across the plant kingdom identified single CYP98 copies in bryophytes, lycophytes, ferns, and gymnosperms, whereas angiosperm genomes often contain more than one gene copy (Alber et al.,

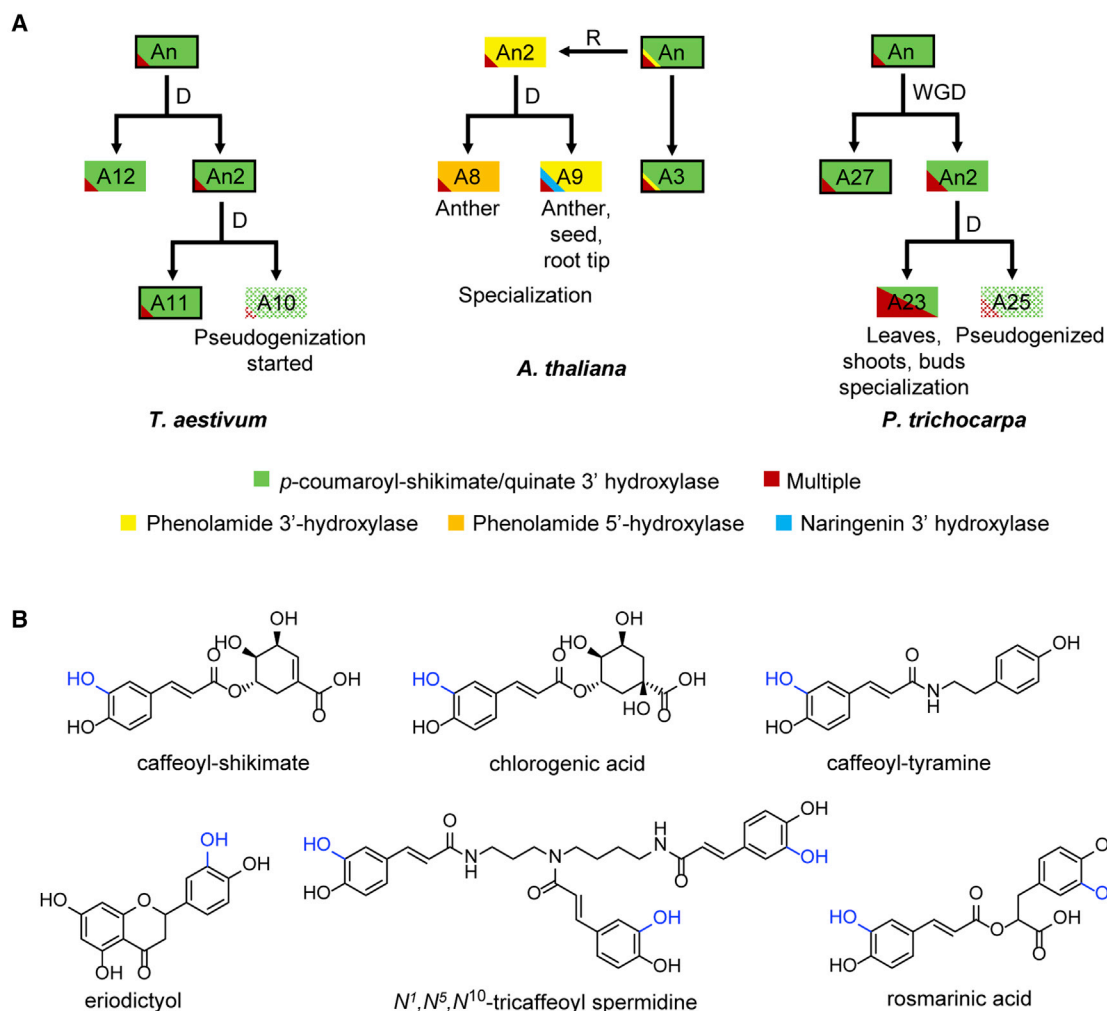


Figure 4. CYP98 gene evolution and functional diversity.

(A) Proposed CYP98 gene duplication and functional divergence in *T. aestivum* (Morant et al., 2007), *A. thaliana* (Matsuno et al., 2009; Liu et al., 2016), and *P. trichocarpa* (Alber et al., 2019). Boxes with a thick frame represent the essential paralog dedicated to the synthesis of lignin precursors and that is expressed in lignified tissues. An, ancestor; D, duplication; R, retrotransposition; WGD, whole-genome duplication.

(B) Products of diverse CYP98 reactions. The introduced hydroxyl groups are colored blue.

2019). Despite absence of lignin in moss, the *P. patens* CYP98 is essential for plant 3D-development. Null mutation of *PpCYP98A34* leads to defects in gametophore development, in the biosynthesis of the major soluble phenolic derivative caffeoyl-threonate, and in the formation of the cuticle polymer (Renault et al., 2017a). Thus, already in early land plants, the canonical CYP98 has been required for plant aerial growth and for the formation of essential biopolymers. Early on, it used the *p*-coumaroyl shikimate ester as a main substrate for phenolic ring *meta*-hydroxylation forming a regulatory shikimate-sensing loop for the biochemical regulation of the lignin pathway (Kriegshauser et al., 2021).

Phylogenetic analysis shows several CYP98 gene duplications within various angiosperm lineages that have occurred independently (Alber et al., 2019). Consistent with relaxed selection on one of the duplicates, promiscuity consistently increases after gene duplication favoring acquisition of new functions. For example, *T. aestivum* (monocot, Poales), *Arabidopsis thaliana*

(eudicot, Brassicales), and *Populus trichocarpa* (eudicot, Malpighiales) all contain three CYP98s, where the duplicate members have arisen independently in the different lineages and where some of the additional CYP98 copies have functionally diversified with distinct outcomes in the different species (Figure 4) (Morant et al., 2007; Matsuno et al., 2009; Alber et al., 2019). The physiological implications for duplicate retention are best understood for *A. thaliana*.

The three *A. thaliana* CYP98s are named CYP98A3, CYP98A8, and CYP98A9. A retrotransposition of the canonical CYP98 occurred early during evolution of Brassicales and a later tandem duplication after divergence of the genus *Aethionema* gave rise to *AtCYP98A8* and *AtCYP98A9* (Matsuno et al., 2009; Liu et al., 2016). *AtCYP98A3* has maintained the original *p*-coumaroyl-shikimate 3'-hydroxylation activity, whereas *AtCYP98A8* and *AtCYP98A9* have functionally diversified and have been recruited to an anther-specific phenolamide pathway to produce di(hydroxyferuloyl)-sinapoylspermidine, which is a major

constituent of the pollen coat and helps in structuring the pollen wall (Matsuno et al., 2009; Xu et al., 2014). In the diversification process, the original shikimate ester hydroxylase activity was lost. Interestingly, the new pathway seems to have emerged from a minor promiscuous activity in the common ancestor still detectable in AtCYP98A3. Homology modeling predicts increased flexibility and enlarged substrate-access channel and active-site cavity in AtCYP98A8 and AtCYP98A9 compared with AtCYP98A3, enabling metabolism of the larger spermidine-conjugated substrates. The production of di(hydroxyferuloyl)-sinapoylspermidine from tri-coumaroyl-spermidine is dependent on both 3'- and 5'-hydroxylations of the three phenol rings. AtCYP98A9 has 3'-hydroxylase activity, whereas AtCYP98A8 possesses 3'- as well as 5'-hydroxylase activities *in vitro*. However, mutant studies show that both CYP98s are necessary to achieve normal levels of di(hydroxyferuloyl)-sinapoylspermidine (Matsuno et al., 2009; Liu et al., 2016). In addition, AtCYP98A9 over-expression lines point to a role in the metabolism of soluble flavonoids, and naringenin 3'-hydroxylase activity was observed with CYP98A9 *in vitro* (Liu et al., 2016). Evolution of the CYP98A8/CYP98A9 cluster within Brassicales is complex: some genera have lost the CYP98A9 ortholog, whereas their CYP98A8 ortholog has maintained dual 3'- and 5'-hydroxylase activity on spermidine conjugates.

In other plant species, duplication and neofunctionalization of CYP98 genes have resulted in the ability to carry out hydroxylations in rosmarinic acid biosynthesis. This applies to CYP98A6 from *Lithospermum erythrorhizon* (Matsuno et al., 2002), CYP98A14 from *Coleus blumei* (Eberle et al., 2009), and CYP98A112 and CYP98A113 from *Phavelia campanularia* (Levsh et al., 2019), all species belong to the Lamiids clade. Phylogenetics combined with biochemical analyses showed that rosmarinic acid biosynthesis has evolved independently at least twice in the Lamiids (Levsh et al., 2019). Hydroxylation at the 3 and 3' position of 4-coumaroyl-4'-hydroxyphenyllactic acid is needed to form rosmarinic acid. Although LeCYP98A14 catalyzes hydroxylation at both the 3 and 3' position (but most efficiently at the 3 position), PcCYP98A112, PcCYP98A113, and CbCYP98A6 carry out monohydroxylations. Although rosmarinic acid is mainly known from species belonging to the Lamiids, the compound sporadically appears throughout land plant phylogeny, as early as hornworts and ferns (Petersen et al., 2009). CYP98 members are present in all the plant lineages currently known to accumulate rosmarinic acid, thus more independent CYP98 specialization events are expected to be associated to the occurrence of rosmarinic acid throughout the plant kingdom.

CYP84

In the series of reactions required for the formation of lignin monomers, a conserved CYP84 (F5H) catalyzes the 5-hydroxylation of coniferaldehyde, the branch point toward S lignin precursors (Meyer et al., 1998; Humphreys et al., 1999). In *A. thaliana*, the 5-hydroxylase CYP84A1 has a paralog, AtCYP84A4, originating from a gene duplication event after the divergence of the *Arabidopsis* genus (Weng et al., 2012). AtCYP84A4 has neofunctionalized to 3-hydroxylate *p*-coumaraldehyde. It forms caffealdehyde and thereby establishes a new branch toward production of arabadopyrones, a group of *Arabidopsis*-

specific compounds. Noteworthy, AtCYP84A1 shows weak *p*-coumaraldehyde 3-hydroxylase activity *in vitro*, although with a *K_m* value more than 100 times higher than for coniferaldehyde (Weng et al., 2010, 2012). The CYP84 family is only present in angiosperms. However, S lignin is also found in the lycopphyte order Selaginellales. In *S. moellendorffii*, a lineage-specific CYP788A1 has been documented to catalyze formation of S lignin via an alternative route (Weng et al., 2008, 2010) (Figure 3). Both CYP84 and CYP788 belong to the 71 clan.

MULTIPLICITY OF SUBSTRATES

Increasing numbers of studies report CYPs with multiple physiological substrates or demonstrate previously overlooked substrate promiscuity. The degree of observed promiscuity is dependent on the number of tested compounds and the range of compound classes tested. Such studies are thus never expected to be exhaustive.

Plant CYP substrate promiscuity can translate into cascade oxidations on a same carbon with various outcomes (e.g., Bak et al., 1997; Helliwell et al., 1999; Cankar et al., 2011), multiple attacks at different positions to catalyze consecutive steps in a same pathway as found in a large diversity of pathways (e.g., Holton et al., 1993; Eberle et al., 2009; Matsuno et al., 2009; Ralston et al., 2001; Bathe et al., 2019; Zabel et al., 2021; Rodriguez-Lopez et al., 2020; Nett et al., 2020), or in multifunctional enzymes sometimes using completely different catalytic mechanisms (Dang et al., 2017; Sibbesen et al., 1995). However, it is most commonly encountered as conversion of multiple natural or man-made compounds showing similar structural and/or physicochemical properties. The latter is usually referred to as “permissiveness” and may be further sub-divided into enzymes utilizing multiple physiological relevant substrates and enzymes being able to metabolize other compounds than its evident physiological relevant substrate. Permissiveness is our main focus in this review since it is considered an important driver of enzyme evolution (Aharoni et al., 2005). The main structural features controlling CYP's permissiveness are illustrated in Figure 5A. It should be mentioned that product promiscuity can be also observed, for example, upon scaffold formation and resulting from intermediate rearrangement and/or associated with the presence of different reductases (e.g., Li et al., 2006; Kellner et al., 2015; Kilgore et al., 2016). Altogether, substrate and product promiscuity allows the formation of complex metabolic networks and production of a series of differently oxygenated molecules with a reduced set of CYPs.

Many terpenoid CYPs show broad substrate range

CYPs metabolizing highly hydrophobic substrates (e.g., terpenoids) have a tendency to show a higher substrate range, possibly because their access channels and active sites are essentially lined by hydrophobic residues and hence are less selective, at least with regard to hydrophobic compounds. This is, for example, illustrated by AtCYP706A3, a CYP with two narrow hydrophobic substrate-access channels and a small hydrophobic substrate-binding site, which was demonstrated to metabolize more than 20 different mono- and sesquiterpenes and their oxidation products, both *in vitro* and in *A. thaliana* flowers as a defense response against florivores (Boachon et al., 2019). Another

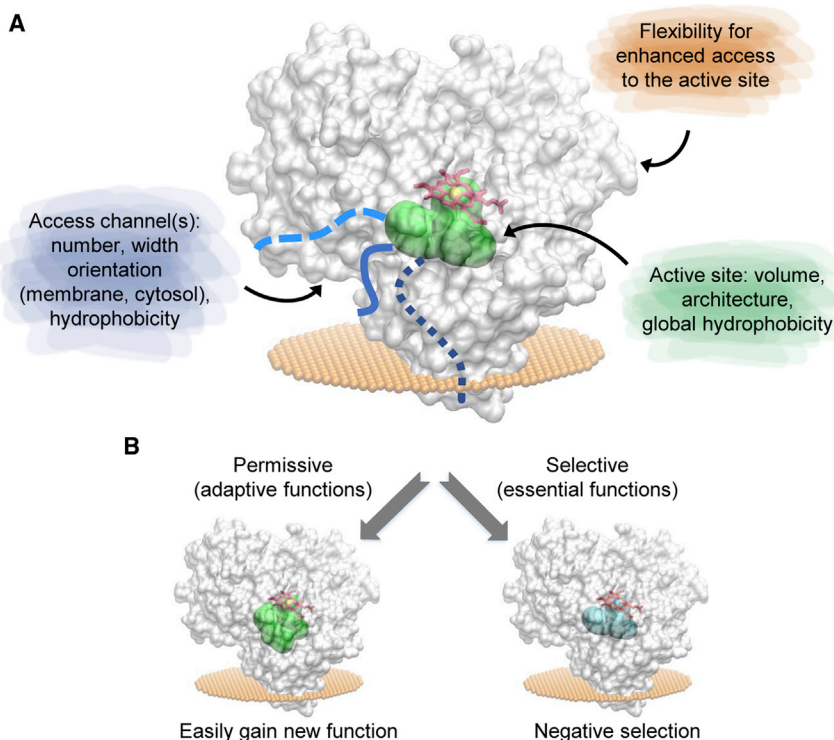


Figure 5. Promiscuity as a driver for evolving new functionalities.

(A) Structural and chemical properties of the active site (green) and the access channel(s) (blue) are determinants of the range of substrates that can be metabolized. Flexible residues located opposite to the main access channel may allow for opening and enhanced access to the active site. New interaction partners and availability of different substrates also contribute to evolution toward new functionalities.

(B) Permissive enzymes are more likely to provide starting points for functional divergences, which may be selected for if they confer an immediate physiological advantage.

example is the multifunctional CYP720B4 from Sitka spruce (*Picea sitchensis*) belonging to the conifer-specific CYP720B subfamily. PsCYP720B4 catalyze consecutive C18 oxidations on eight diterpene backbones when expressed in yeast, generating products that are also observed in the diterpenoid profile of *P. sitchensis* (Hamberger et al., 2011).

Promiscuous tendencies are also observed for CYPs with conserved functions. They can be found in the phenolic pathways as mentioned above for AtCYP98A3 and AtCYP84A1, but are also reported for hormonal metabolism. *Ent*-kaurene oxidases (KO, CYP701 members) oxidize *ent*-kaurene at C19 to form kaurenoic acid in the pathway toward gibberellins, a function that is conserved across land plants (Miyazaki et al., 2011; Nelson and Werck-Reichhart, 2011). Testing of multiple substrates for *A. thaliana* CYP701A3 (AtKO) and *O. sativa* CYP701A6 (OsKO2) showed that AtCYP701A3 can oxygenate several labdane-related diterpene substrates, although with lower affinity and efficiency than for *ent*-kaurene, whereas OsCYP701A6 is more specific for *ent*-kaurene and *ent*-isokaurene (Morrone et al., 2010; Mafu et al., 2016). It was speculated that the metabolic background of labdane-related diterpenes in *O. sativa* played a role in determining the specificity of OsCYP701A6 for this conserved biosynthetic step (Mafu et al., 2016). *O. sativa* harbors five CYP701A genes. One of the additional copies, CYP701A8 (OsKOL4), has diversified to hydroxylate the C3 of the diterpenes sandaracopimaradiene and cassadiene, which are expected intermediates in the biosynthesis of oryzalexin and phytocassane phytoalexins, respectively (Wang et al., 2012b; Wu et al., 2013). CYP701A8 is still able to metabolize *ent*-kaurene, maintaining some promiscuity after neofunctionalization.

Multisubstrate functionalities can shape metabolism as non-linear biosynthetic pathways, as illustrated by CYP76AHs in

forskolin biosynthesis in *Plectranthus barbatus* (often referred to as *Coleus forskohlii*) (Pateraki et al., 2017), or as an hour-glass-shaped biosynthetic network as reported in maize (*Zea mays*) involving CYP71Zs and CYP81As (Ding et al., 2020). In the latter case, three tandemly associated CYP71Z genes form a resilient cluster encoding versatile enzymes that contribute to several sesquiterpenoid and diterpenoid defense pathways (zealexins, kauralexins, dolabrallexins and costic acid), mainly forming carboxylic acids (Mafu et al., 2018; Ding et al., 2019, 2020). A different cluster of three genes codes for permissive CYP81A enzymes catalyzing diverse oxygenation and desaturation reactions more focused on sesquiterpene acidic derivatives to generate the diversity of zealexins (Ding et al., 2020). Permissiveness thereby allows for formation of an efficient, diversified arsenal of defensive compounds.

CYP promiscuity as a seed for new functions

Promiscuity may facilitate the ability of plants to adapt to environmental challenges such as the formation of deterrents to new herbivores and pests and thereby may represent an evolutionary advantage. For these reasons, promiscuous CYPs can be speculated to show faster functional diversification to acquire new specialized functions, with promiscuous activities being selected for when they provide a physiological advantage (Figure 5B). In this context, exploring promiscuity of CYPs can provide insight into underlying mechanisms for evolution of phytochemical profiles. Examples are provided by the above-mentioned CYP98 enzymes in the phenolic metabolism, but further examples can be found in other pathways.

Sesquiterpene lactones are characteristic metabolites in Asteraceae. The most well known, artemisinin from *Artemisia annua*, is species specific and used as an anti-malarial drug. In contrast, costunolide appeared earlier in evolution and is widespread in Asteraceae. In costunolide biosynthesis, germacrene A oxidases (GAOs, CYP71AV members) catalyze the multistep conversion of germacrene A to germacrene A acid (Nguyen et al., 2010, 2019; Cankar et al., 2011; Eljonaoui et al., 2014), while amorphaadiene synthase (CYP71AV1) catalyzes the equivalent step in

artemisinin biosynthesis (Ro et al., 2006; Teoh et al., 2006). Investigation of substrate promiscuity of GAOs showed that they can convert several sesquiterpenes, including amorphadiene, to the corresponding acids, which suggests that amorphadiene synthase activity was embedded in an ancestral GAO holding the catalytic plasticity for gaining new physiological functions (Nguyen et al., 2010, 2019; Cankar et al., 2011; Eljonaïdi et al., 2014). Conversely, the recently evolved species-specific CYP71AV1 from *A. annua* gained more stringent specificity when recruited for a distinct metabolic pathway.

CYP promiscuity can also provide dual functionalities and favor the emergence of herbicide resistance. This is illustrated by the above-mentioned AtCYP706A3. This permissive CYP, the physiological function of which is to reduce the emission of volatile terpenoids in *A. thaliana* and to generate terpene oxides to protect flowers from florivores, also metabolizes volatile and hydrophobic herbicides from the class of dinitroanilines. As a result, shifting the expression of AtCYP706A3 from flowers to the vegetative tissues confers an increased tolerance to dinitroanilines (Abdollahi et al., 2021). A similar dual functionality has been observed for promiscuous monoterpenol metabolizing CYP76s that confer a tolerance to another class of herbicides, the phenylureas (Höfer et al., 2014). In this light, it is striking that members of the CYP81A subfamily have been recently shown to metabolize a broad range of herbicides, thereby conferring herbicide resistance to monocotyledonous weeds (Dimaano and Iwakami, 2020; Han et al., 2020), while CYP81As from maize were demonstrated to catalyze diverse oxidation reactions on acidic sesquiterpenes to form zealexin antibiotics (Ding et al., 2020).

REPEATED RECRUITMENT OF CYPS FROM THE SAME FAMILY

Several compounds show a patchy distribution across plant phylogeny, and multiple examples of parallel evolution are known where plants independently have acquired the ability to produce the same compound. Some CYPs with the same catalytic function are not orthologs and CYPs may be independently and repeatedly recruited from the same CYP family (discussed here) or from diverse CYP families (discussed in the next section) to catalyze the same reaction. Parallel evolution involving CYPs from the same family have already been exemplified by the CYP98 and the CYP74 families, and more examples are discussed below.

Flavonoid 3′/5′-hydroxylating CYP75

Several members of the CYP75 family hydroxylate the B ring of flavonoids at the 3′ position (F3′H) or catalyze hydroxylation at both the 3′ and 5′ positions (F3′/5′H). Most F3′/5′Hs belong to the CYP75A subfamily, but some diverse CYP75B members, which are usually F3′H, have independently gained 5′-hydroxylase activity (Seitz et al., 2006, 2015; Lui et al., 2020). F3′/5′H activity has thus evolved from F3′H activity several times. Specific amino acids in especially SRS6 have been identified to be important for the 5′-hydroxylase activity (Seitz et al., 2007, 2015; Lui et al., 2020). Pollinator attraction has been suggested to be a driving force behind gain of F3′/5′H activity as it affords production of purple to blue anthocyanins in flower petals (Seitz et al., 2006, 2015).

Amino acid-metabolizing CYP79

All functionally characterized CYP79 family members catalyze a multistep reaction converting an amino acid (or in some cases a chain-elongated amino acid) into a corresponding oxime (Kahn et al., 1999; Sibbesen et al., 1995). The catalysis proceeds via two consecutive *N*-hydroxylations followed by a dehydration and decarboxylation (Gleadow and Møller, 2014). The oximes can be released as volatiles (Irmisch et al., 2013a, 2013b; Luck et al., 2016) or further metabolized to, for example, glucosinolates or cyanogenic glucosides (Gleadow and Møller, 2014) (Figure 6A). In spite of the complex catalytic process catalyzed by the CYP79s, phylogenetic studies demonstrate that substrate preference toward specific amino acids cannot be assigned to separate subfamilies. Likewise, the CYP79s giving rise to oximes used for cyanogenic glucoside biosynthesis are scattered throughout the CYP79 phylogeny and cannot be identified based solely on phylogenetic analyses. For example, CYP79A125 from *Eucalyptus cladocalyx* and CYP79D16 from *Prunus dulcis* belong to different CYP subfamilies but both catalyze the conversion of phenylalanine into phenylacetaldoxime in cyanogenic glucoside biosynthesis (Hansen et al., 2018; Thodberg et al., 2018). A few changes in a CYP79 amino acid sequence is sometimes enough to change substrate specificity. A study investigating glucosinolate accumulation in two *Boechera stricta* populations with distinct chemical profiles identified two amino acid positions (134 and 536) in glucosinolate CYP79Fs that showed accelerated evolution (Prasad et al., 2012). Individually, as well as in combination, the G134L and P536K mutations enabled the mainly methionine-metabolizing BsCYP79F4v1 (BCMA2) to increase conversion of valine *in vitro*. In one of the *B. stricta* populations, the closely related BsCYP79F3 (BCMA1-MT) had also acquired the ability to metabolize isoleucine. Many CYP79s are specific toward a certain amino acid *in planta*, but sometimes show promiscuous substrate abilities when assayed *in vitro*. In contrast, some CYP79Ds from *Populus* species metabolize a few amino acids, and the corresponding aldoximes are present in the volatile blend of the species (Irmisch et al., 2013a, 2013b). These examples illustrate the functional plasticity of the CYP79 family. Some modern ferns produce cyanogenic glucosides, but genes encoding CYP79s are not present in this taxonomic group. Instead, a flavin-containing monooxygenase was recently found to convert phenylalanine to phenylacetaldoxime in the ferns *P. aureum* and *Pteridium aquilinum* (Thodberg et al., 2020).

Terpenoid CYP76

CYP76 is one of the families characterized by multiple duplications and losses in different lineages illustrated by a large number of lineage-specific subfamilies and resulting in completely different subfamily sets in major lineages (e.g., gymnosperms, dicots, or monocots) (Figure 7). This bursting protein diversification translates into a number of lineage-specific pathways, such as phytocassane and oryzalexin classes of diterpenoid phytoalexins in monocots (CYP76M subfamily; Wang et al., 2012a), labdane-type diterpenoids in Lamiales (CYP76AH/AK subfamilies; Scheler et al., 2016), or tyrosine-derived betalain pigments in Caryophyllales (CYP76AD subfamily; Timoneda et al., 2019). Within dicots, emergence of new CYP76 subfamilies is repeatedly associated with species-specific adaptive functions of monoterpenol

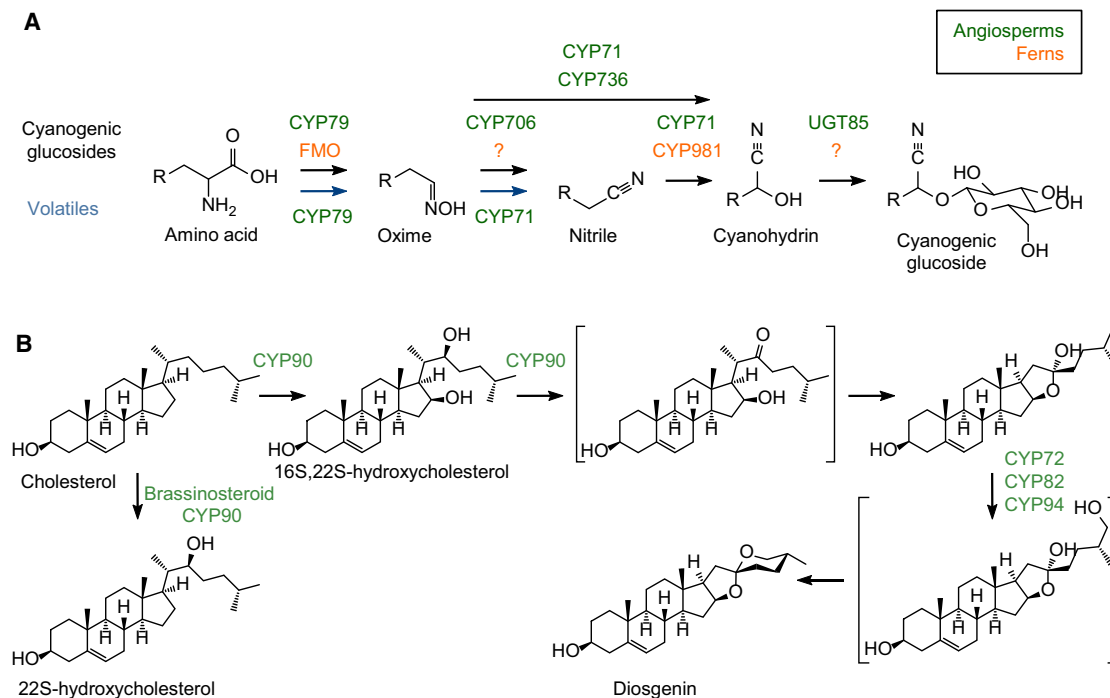


Figure 6. Identical or similar CYP reactions catalyzed by diverse CYP families.

(A) Biosynthetic pathway for production of amino acid-derived oximes, nitriles, and cyanogenic glucosides.

(B) Suggested diosgenin biosynthesis according to Christ et al. (2019). Another possible intermediate toward the first ring formation could also be a dihydroxylation of C22.

metabolism (e.g., Höfer et al., 2014; Miettinen et al., 2014; Ilc et al., 2016). So far, CYP76s from the subfamilies C, B, F, and T were found involved in the formation of oxygenated monoterpene derivatives, catalyzing cascade oxygenations of linalool, geraniol, and other monoterpenols (Collu et al., 2001; Wang et al., 2010; Ginglinger et al., 2013; Boachon et al., 2015; Ilc et al., 2017). For example, the lineage-specific CYP76C1 from *A. thaliana* and CYP76F14 from *V. vinifera* both oxygenate linalool to 8-carboxylinalool via the intermediates 8-hydroxylinalool and 8-oxolinalool (Boachon et al., 2015; Ilc et al., 2017) as a result of parallel evolution.

Steroid hydroxylating CYP90

The spiroketal steroid diosgenin is sporadically distributed in monocots and dicots. A comparison of diosgenin biosynthesis in *Paris polyphylla* (monocot, Liliales) and *Trigonella foenum-graecum* (dicot, Fabales) shows that the CYPs have been independently recruited for the biosynthesis of diosgenin from cholesterol (Christ et al., 2019). The biosynthesis is initiated by two hydroxylations at C16 and C22 followed by a cyclization. *Pp*CYP90G4 and *Tf*CYP90B50 arose from the canonical brassinosteroid CYP90s by two independent gene duplications events, and both catalyze 16,22-dihydroxylation (Figure 6B). Investigation of the brassinosteroid C22-hydroxylases, *Pp*CYP90B52 and *Tf*CYP90B51, by transient expression in *N. benthamiana*, revealed formation of minor amounts of 16,22-dihydroxylated cholesterol, suggesting that minor side-activity seeded repeated emergence of the dihydroxylase activity for diosgenin biosynthesis. Mutation of nine amino acid residues that were differentially conserved between the diosgenin CYP90s and brassinosteroid CYP90s resulted in increased 16,22-dihydroxylase activity but did not obliterate

C22-monohydroxylation activity of the brassinosteroid CYP90s (Christ et al., 2019).

REPEATED RECRUITMENT OF CYPs FROM DIFFERENT FAMILIES

While repeated recruitment of CYPs from the same family has occurred for the first multistep reaction in cyanogenic glucoside and diosgenin biosynthesis, CYPs from distinct families have been recruited for the downstream biosynthetic steps. Other compounds with scattered distribution across plant phylogeny also demonstrate recruitment of distinct CYP families for catalysis of identical biosynthetic steps.

Cyanogenic glucosides and volatile nitriles

In cyanogenic glucoside biosynthesis, the amino acid-derived oxime produced by a CYP79 (or an FMO in ferns) is converted into an α -hydroxynitrile, which is subsequently glucosylated to produce the cyanogenic glucoside (Gleadow and Möller, 2014). In several cyanogenic plant species, conversion of the oxime to the α -hydroxynitrile is catalyzed by a multifunctional CYP71 (Bak et al., 1998; Kahn et al., 1997) (Figure 6A). Nomenclature-wise, such a CYP may also be named a CYP83. Similar to the CYP79s, the CYP71s involved in cyanogenic glucoside biosynthesis do not form a monophyletic group. In *L. japonicas*, a CYP from a distinct family, CYP736A2, catalyzes the multistep conversion of two aliphatic oximes to the corresponding α -hydroxynitriles (Talos et al., 2011). In *E. cladocalyx*, two CYPs have been recruited to complete the catalytic sequence of reactions to form an α -hydroxynitrile from an oxime (Hansen et al., 2018). First, *Ec*CYP706C55 converts phenylacetaldoxime to

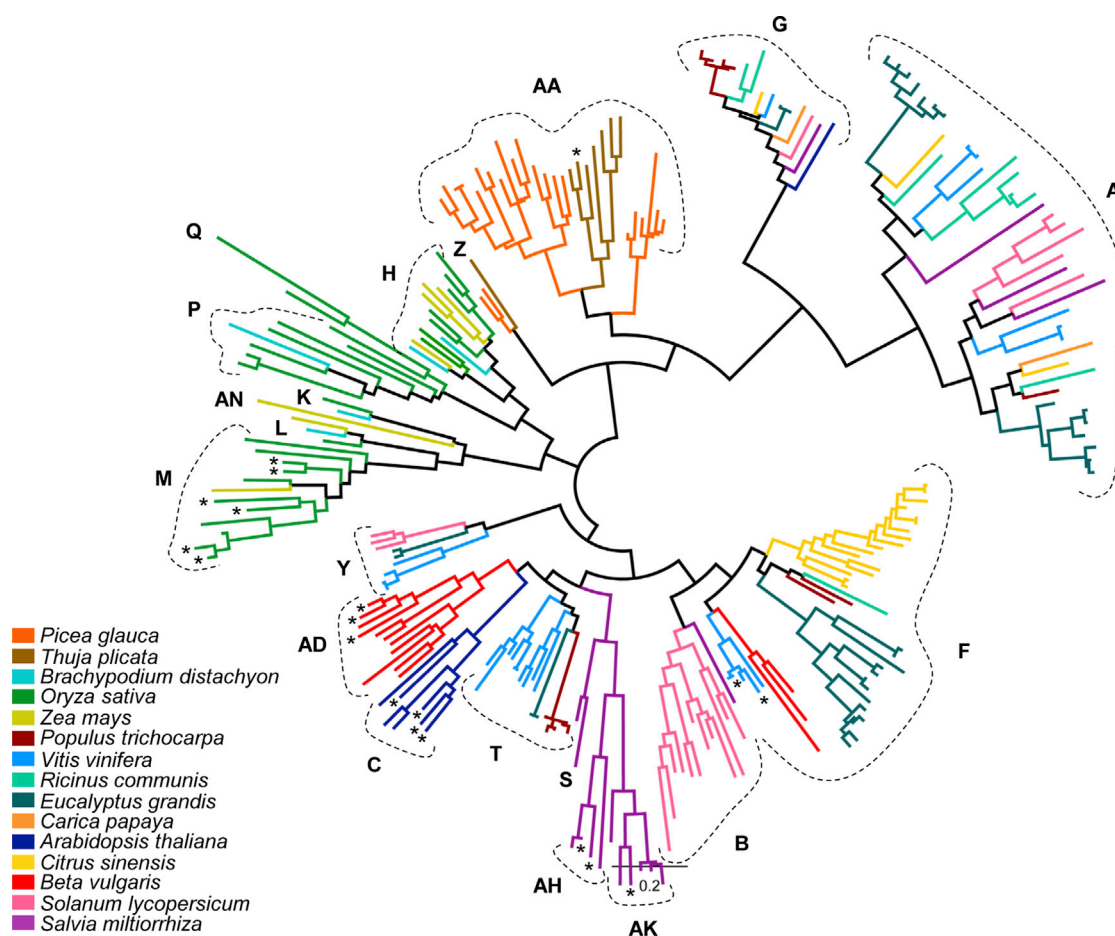


Figure 7. Dynamics of the CYP76 family.

Subfamily CYP76C is specific to Brassicales, CYP76AH/AK is specific to Lamiales, CYP76AD is specific to Caryophyllales, and subfamily CYP76AA is specific to gymnosperms. Subfamily CYP76M is specific to monocots and has particularly expanded in *O. sativa* (green), *T. aestivum* and *Saccharum officinarum*. Subfamily CYP76F members are present in several angiosperm species but have particularly expanded in *Citrus sinensis* and *E. grandis*. In contrast, subfamily CYP76G tend to be maintained at a single or low copy number in most rosids and asterids. CYP76s marked by an asterisk are functionally characterized: AtCYP76C1 (Höfer et al., 2014; Boachon et al., 2015), AtCYP76C2 (Höfer et al., 2014), AtCYP76C3 (Ginglinger et al., 2013), AtCYP76C4 (Höfer et al. 2013; Höfer et al., 2014), VvCYP76F12 and VvCYP76F14 (Ilc et al., 2017), OsCYP76M2 (Wang et al., 2012a), OsCYP76M5 (Wang et al., 2012a), OsCYP76M6 (Wang et al., 2012a; Wu et al., 2013), OsCYP76M7 (Swaminathan et al., 2009), OsCYP76M8 (Wang et al., 2012a; Wu et al., 2013), OsCYP76M10 (Wang et al., 2012a), TpcYP76AA25 (Gesell et al., 2015), BvCYP76AD1 (Hatlestad et al., 2012), BvCYP76AD5 (Sunnadeniya et al., 2016), BvCYP76AD6 (Polturak et al., 2016); (Sunnadeniya et al., 2016), SmCYP76AH1 ((Guo et al., 2013); (Zi and Peters 2013), SmCYP76AH3 (Guo et al., 2016), and SmCYP76AK1 (Guo et al., 2016). All full-length CYP76 sequences from the listed species have been used to generate the tree. Amino acid sequences were aligned using MUSCLE and phylogeny was inferred by maximum likelihood analysis. A phylogenetic tree with tip labels is available in supplemental Figure 2.

phenylacetonitrile, which is subsequently hydroxylated by *EcCYP71B103* to form the α -hydroxynitrile mandelonitrile. In the fern *P. aureum*, CYP981F5 was recently identified to hydroxylate phenylacetonitrile in cyanogenic glucoside biosynthesis (Thodberg et al., 2021). *P. trichocarpa* and *Fallopia sachalinensis* also produce phenylacetonitrile, which is released upon herbivore attack (Irmisch et al., 2014; Yamaguchi et al., 2016). Conversion of phenylacetaldoxime to phenylacetonitrile is catalyzed by CYP71B40v3 and CYP71B41v2 in *P. trichocarpa* (Irmisch et al., 2014) and by CYP71AT96 in *F. sachalinensis* (Yamaguchi et al., 2016). Numerous members of the large CYP71 family have been characterized, and this family covers a wide range of substrates and catalytic functions, some of which are also referred to in this review. Fewer members of the CYP736 and CYP706 families have been functionally

characterized, but it is striking that both families show activities toward small, often volatile compounds. CYP736 functions are mentioned above and the CYP706 family oxygenates various monoterpenes, sesquiterpenes, and flavonoids (Luo et al., 2001; Cankar et al., 2014; Liu et al., 2018; Boachon et al., 2019).

Specialized steroids

In diosgenin biosynthesis, the step after the *PpCYP90G4/TtCYP90B50* hydroxylations and subsequent cyclization is an end-of-tail-hydroxylation followed by another cyclization to form the spiroketal moiety. *P. polyphylla* and *T. foenum-graecum* have recruited various CYP families for this purpose (Christ et al., 2019). In *P. polyphylla*, diosgenin production was achieved by the combined action of CYP90G4 with CYP94D108, CYP94D109, or

CYP72A616. In *T. foenum-graecum*, CYP90B50 together with either CYP82J17 or CYP72A613 catalyzed diosgenin formation (Figure 6B). *PpCYP90G4* co-expressed with *PpCYP94D108* and *TfCYP90B50* co-expressed with *TfCYP82J17* resulted in the highest diosgenin production as monitored by transient expression in *N. benthamiana*. The CYP94 family is known to metabolize jasmonate conjugates essential for jasmonic acid regulation (e.g., Heitz et al., 2012; Widemann et al., 2015) but is also reported as having activity toward other hydrophobic substrates (Tijet et al., 1998; Le Bouquin et al., 1999; Augustin et al., 2015). The CYP82 family shows broad catalytic activities toward various flavones, coumarins, alkaloids, and terpenes (e.g., Lee et al., 2010; Dang et al., 2017; Zhao et al., 2018b; Rajniak et al., 2018). Several CYP72 members are known to metabolize hydrophobic isoprene-derived compounds such as steroloids, other triterpene backbones, and diterpenes (e.g., Umemoto et al., 2016; He et al., 2019; Liu et al., 2019), and also to catalyze various hydroxylation, oxidation, and ring-opening steps leading to iridoid derivatives in indole alkaloid biosynthesis (Irmiler et al., 2000; Salim et al., 2013; Yang et al., 2019; Rodriguez-Lopez et al., 2020).

Furanocoumarins

Furanocoumarins are tricyclic phenolic derivatives present in a few non-related plant families (Pathak et al., 1962). Hydroxylation of xanthotoxin to 5-hydroxy-xanthotoxin in the biosynthesis of the linear furanocoumarin isopimpinellin is catalyzed by CYP71AZ1 in *Ammi majus* and CYP71AZ6 in parsnip (*Pastinaca sativa*; both Apiaceae family) (Krieger et al., 2018), whereas CYP82A64 catalyzes the same reaction in *Citrus paradisi* (Rutaceae) (Limonés-Mendez et al., 2020). Both CYP families cover broad catalytic activities, as mentioned earlier in the review.

Momilactones

Momilactone diterpenoids are well-known phytoalexins from *O. sativa* but are also reported to occur in the mosses *C. plumiforme* and *Pseudoleskeella papillosa* (Zhao et al., 2018a). CYPs involved in momilactone biosynthesis have been identified for *O. sativa* (Shimura et al., 2007; Wang et al., 2011; De La Pena and Sattely, 2020) and *C. plumiforme* (Mao et al., 2020). In *O. sativa*, CYP99A3 catalyzes consecutive oxygenations of the momilactone precursor *syn*-pimaradiene at the C19 methyl group to form *syn*-pimaradienoic acid (Wang et al., 2011), and CYP76M8 has been shown to hydroxylate multiple labdane diterpenes, including C6-hydroxylation of *syn*-pimaradiene (Wang et al., 2012a). A more recent study co-expressed the terpene synthases OsKSL4 and OsCPS4 together with OsCYP99A3 and OsCYP76M8 in *N. benthamiana* and observed accumulation of *syn*-pimaradiene with oxidations at C6 and/or C19, some of which might be catalyzed by endogenous *N. benthamiana* oxidases (De La Pena and Sattely, 2020). However, co-expression of each of the OsCYPs with the terpene synthases confirmed previously reported OsCYP activities on *syn*-pimaradiene. Co-expression of the dehydrogenase OsMAS with the two terpene synthases and two CYPs in *N. benthamiana* resulted in formation of the lactone ring. Accumulation of momilactone A and small amounts of other oxidation products were also observed. Other CYP-catalyzed steps required for momilactone production are oxygenation at C3 and C20. Further addition of C20-hydroxylating OsCYP76M14 and

the promiscuous OsCYP701A8 resulted in production of momilactone B. Another recent study showed involvement of OsCYP99A3, OsCYP76M8, and OsCYP701A8 in production of momilactone A using engineered *Escherichia coli* and *in vitro* assays (Kitaoka et al., 2021). In *C. plumiforme*, moss-specific CYP families have been recruited for momilactone production. CpCYP970A14 (71 clan) converts *syn*-pimaradiene to *syn*-pimaradienoic acid similarly to OsCYP99A3, and CpCYP964A1 (85 clan) was found to hydroxylate C3 of *syn*-pimaradiene (Mao et al., 2020). Co-expression of the CpCYPs in *N. benthamiana* together with the bifunctional terpene synthase CpDTC resulted in 3OH-*syn*-pimaradienolide and some momilactone A, the latter compound possibly resulting from endogenous *N. benthamiana* enzyme activity. Interestingly, in both plant species, the genes encoding biosynthetic enzymes for momilactone production are localized within gene clusters (Shimura et al., 2007; Mao et al., 2020; Kitaoka et al., 2021).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In this review, we highlight the immense numbers and functionalities of CYPs present in the plant lineage. While reports on functional characterization of CYPs from seed plants have increased dramatically in recent years, some families, including some of the most conserved, remain functionally uncharacterized. Their deorphanization is expected to reveal new branches in plant hormone pathways and possibly overlooked classes of signaling compounds. Moreover, only a very few CYPs from more recently sequenced green algae and basal land plant species have been investigated, which leaves much to explore to fully apprehend the diversity of plant CYP activities.

The current surge of open access sequence information and rise in transcriptome and genome sequence data provides new opportunities to understand the molecular events leading to the emergence of new functions. Most notably, the number of sequenced genomes from extant charophytes keeps increasing (Hori et al., 2014; Nishiyama et al., 2018; Cheng et al., 2019; Wang et al., 2020b; Jiao et al., 2020) and now offers the opportunity to disclose events resulting in the evolution of land plants in context with the acquisition of CYPs with new functionalities. In addition, useful information on metabolic pathways involving orphan CYPs is expected to arise from parallel analyses with other enzyme families (e.g., terpene synthases, glycosyltransferases, dehydrogenases, methyl transferases).

Beyond catalytic activities, CYP's physiological functions are often not investigated or are limited to studies of specific facets of their roles in plant development, growth, or fitness. All aspects of plant ontogeny and the exposures to varying growth conditions, abiotic and biotic (negative and positive) conditions, ecological niches, including metabolism and interference of man-made exogenous compounds such as pollutants and pesticides, need to be considered to integrate the complex role of CYPs in different ecological systems with plant metabolic networks and physiology.

A previously largely overlooked characteristic emerging within the last years is the promiscuity of some plant CYP enzymes.

This feature clearly favors functional diversification and emergence of new functions in gene duplicates. Because duplications result in relaxation of substrate specificity, it also clearly relates to the evolution of blooms within some CYP families playing an important role in the flexibility, adaptability, and resilience of plants' specialized metabolism. Thorough exploration of individual plant CYP substrate ranges is expected to unravel additional functionalities, and resulting overlaps or crosstalks between pathways and adaptive functions. Such knowledge would also obviously serve to expand the use of CYPs in synthetic biology approaches aimed at developing environmentally benign production systems for the future (Møller, 2017).

In addition to the structures of atypical CYP74s, the crystal structures of five plant CYP proteins representative of three clans are now accessible in public repository (Fujiyama et al., 2019; Gu et al., 2019; Niu et al., 2020; Zhang et al., 2020). This is an important asset for modeling other CYP protein structures, so as to understand ligand binding in the active sites and to engineer CYP activities. With the availability of an increasing number of sequences, reference structures, and characterized activities, it is now possible to envisage modeling approaches to predict CYP substrates and activities. Systematic studies taking into consideration the molecular fingerprint of compounds and the sequences of the SRSs may also be used to predict substrate candidates for CYP families for which no substrate has yet been found. However, it is necessary to keep in mind that, in addition to the physiological substrate(s), additional compounds may gain significant docking scores. In this context, the role of the access channel in the filtration of potential substrates is still poorly understood and has to be further considered.

Other central questions to be explored regard the importance of substrate(s) availability in the plant tissue for directing fluxes; possible diverging activities in different tissues; supramolecular organization of the pathway enzymes, including how this organization compartmentalizes or interconnects different pathways; and regulatory factors for driving metabolic fluxes.

In conclusion, the multiplicity of CYPs in plants in combination with their functionality and promiscuity provides an excellent molecular background for development of metabolic diversity and plasticity required by plants to adapt to different and rapidly changing growth conditions and environmental challenges. More studies into the substrate space of individual CYPs may provide essential knowledge to understand the evolution-specific functional characteristics. Knowledge of enzyme promiscuity may also guide engineering of desired specificities for, e.g., optimized biotechnological applications, and may help to predict and manage metabolism of pesticides, other encountered exogenous chemicals, and pollutants. We hope that The Plant Cytochrome P450 Database will facilitate search for hitherto functionally characterized CYPs for further investigations, biotechnological applications, and the discovery of CYPs with unknown functions.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Molecular Plant Online*.

FUNDING

C.C.H. is supported by a PhD fellowship provided through a Villum Foundation Young Investigator Program fellowship granted to Elizabeth H.J.

Neilson (grant number 13167). This work was supported by the VILLUM Center for Plant Plasticity (VKR023054) (B.L.M.); a European Research Council Advanced Grant (ERC-2012-ADG_20120314), and the Novo Nordisk Foundation Distinguished Investigator 2019 Grant (NNF 0054563, The Black Holes in the Plant Universe). B.L.M. acknowledges the continued strong external support from Danish Foundations and the European Research Council throughout his career.

AUTHOR CONTRIBUTIONS

C.C.H., D.W.R., and B.L.M. contributed to conceptualization. D.N. provided quantitative data on CYP families, compiled the list of plant CYP amino acid sequences, and constructed the large green algae and land plant CYP phylogeny. C.C.H. compiled The Plant Cytochrome P450 Database. C.C.H. outlined the manuscript and prepared the first draft and figures. All authors contributed with inputs and revisions.

ACKNOWLEDGMENTS

We thank Drs. Charles Francis Delwiche and Endymion Cooper for early access to green algal transcriptomes supported by NSF, grant DEB-1036506. We also thank Dr. Elizabeth H.J. Neilson for comments on early versions of the manuscript, Dr. François André for providing the protein model structures in Figure 5, and Jakob Bo Cetti Hansen for help coding The Plant Cytochrome P450 Database. The authors declare no competing interests.

Received: March 24, 2021

Revised: May 28, 2021

Accepted: June 30, 2021

Published: June 30, 2021

REFERENCES

- Abdollahi, F., Alebrahim, M.T., Ngov, C., Lallemand, E., Zheng, Y., Villette, C., Zumsteg, J., André, F., Navrot, N., Werck-Reichhart, D., et al. (2021). Innate promiscuity of the CYP706 family of P450 enzymes provides a suitable context for the evolution of dinitroaniline resistance in weed. *New Phytol.* **229**:3253–3268.
- Abe, S., Sado, A., Tanaka, K., Kisugi, T., Asami, K., Ota, S., Kim, H.I., Yoneyama, K., Xie, X., Ohnishi, T., et al. (2014). Carlactone is converted to carlactonoic acid by MAX1 in *Arabidopsis* and its methyl ester can directly interact with AtD14 in vitro. *Proc. Natl. Acad. Sci. U S A* **111**:18084–18089.
- Aharoni, A., Gaidukov, L., Khersonsky, O., Gould, S.M., Roodveldt, R., and Tawfik, D.S. (2005). The 'evolvability' of promiscuous protein functions. *Nat. Genet.* **37**:73–76.
- Akashi, T., Aoki, T., and Ayabe, S.-I. (1999). Cloning and functional expression of a cytochrome P450 cDNA encoding 2-hydroxyisoflavanone synthase involved in biosynthesis of the isoflavanoid skeleton in licorice. *Plant Phys.* **121**:821–828.
- Alber, A.V., Renault, H., Basilio-Lopes, A., Bassard, J.E., Liu, Z., Ullmann, P., Lesot, A., Bihel, F., Schmitt, M., Werck-Reichhart, D., et al. (2019). Evolution of coumaroyl conjugate 3-hydroxylases in land plants: lignin biosynthesis and defense. *Plant J.* **99**:924–936.
- Augustin, M.M., Ruzicka, D.R., Shukla, A.K., Augustin, J.M., Starks, C.M., O'Neil-Johnson, M., McKain, M.R., Evans, B.S., Barrett, M.D., Smithson, A., et al. (2015). Elucidating steroid alkaloid biosynthesis in *Veratrum californicum*: production of verazine in Sf9 cells. *Plant J.* **82**:991–1003.
- Bak, S., Beisson, F., Bishop, G., Hamberger, B., Hofer, R., Paquette, S., and Werck-Reichhart, D. (2011). Cytochromes p450. *Arabidopsis Book* **9**:e0144.
- Bak, S., Kahn, R.A., Nielsen, H.L., Møller, B.L., and Halkier, B.A. (1998). Cloning of three A-type cytochromes P450, CYP71E1, CYP98, and CYP99 from *Sorghum bicolor* (L.) Moench by a PCR approach and identification by expression in *Escherichia coli* of CYP71E1 as a

- multifunctional cytochrome P450 in the biosynthesis of the cyanogenic glucoside dhurrin. *Plant Mol. Biol.* **36**:393–405.
- Bak, S., Kahn, R.A., Olsen, C.E., and Halkier, B.A.** (1997). Cloning and expression in *Escherichia coli* of the obtusifoliiol 14 α -demethylase of *Sorghum bicolor* (L.) Moench, A cytochrome P450 orthologous to the sterol 14 α -demethylases (CYP51) from fungi and mammals. *Plant J.* **11**:191–201.
- Bathe, U., Frolov, A., Porzel, A., and Tissier, A.** (2019). CYP76 oxidation network of abietane diterpenes in Lamiaceae reconstituted in yeast. *J. Agric. Food Chem.* **67**:13437–13450.
- Becker, B.** (2013). Snow ball earth and the split of Streptophyta and Chlorophyta. *Trends Plant Sci.* **18**:180–183.
- Bicalho, K.U., Santoni, M.M., Arendt, P., Zanelli, C.F., Furlan, M., Goossens, A., and Pollier, J.** (2019). CYP712K4 catalyzes the C-29 oxidation of friedelin in the *Maytenus ilicifolia* quinone methide triterpenoid biosynthesis pathway. *Plant Cell Physiol.* **60**:2510–2522.
- Boachon, B., Burdloff, Y., Ruan, J.X., Rojo, R., Junker, R.R., Vincent, B., Nicole, F., Bringel, F., Lesot, A., Henry, L., et al.** (2019). A promiscuous CYP706A3 reduces terpene volatile emission from *Arabidopsis* flowers, affecting florivores and the floral microbiome. *Plant Cell* **31**:2947–2972.
- Boachon, B., Junker, R.R., Miesch, L., Bassard, J.E., Hofer, R., Caillieudeaux, R., Seidel, D.E., Lesot, A., Heinrich, C., Ginglinger, J.F., et al.** (2015). CYP76C1 (cytochrome P450)-mediated linalool metabolism and the formation of volatile and soluble linalool oxides in *Arabidopsis* flowers: a strategy for defense against floral antagonists. *Plant Cell* **27**:2972–2990.
- Bozak, K.R., Yu, H., Sirevåg, R., and Christoffersen, R.E.** (1990). Sequence analysis of ripening-related cytochrome P-450 cDNAs from avocado fruit. *Proc. Natl. Acad. Sci. U S A* **87**:3904–3908.
- Cankar, K., van Houwelingen, A., Bosch, D., Sonke, T., Bouwmeester, H., and Beekwilder, J.** (2011). A chicory cytochrome P450 monooxygenase CYP71AV8 for the oxidation of (+)-valencene. *FEBS Lett.* **585**:178–182.
- Cankar, K., van Houwelingen, A., Goedbloed, M., Renirie, R., de Jong, R.M., Bouwmeester, H., Bosch, D., Sonke, T., and Beekwilder, J.** (2014). Valencene oxidase CYP706M1 from Alaska cedar (*Callitropsis nootkatensis*). *FEBS Lett.* **588**:1001–1007.
- Cannell, N., Emms, D.M., Hetherington, A.J., MacKay, J., Kelly, S., Dolan, L., and Sweetlove, L.J.** (2020). Multiple metabolic innovations and losses are associated with major transitions in land plant evolution. *Curr. Biol.* **30**:1783–1800.e11.
- Celedon, J.M., Chiang, A., Yuen, M.M., Diaz-Chavez, M.L., Madilao, L.L., Finnegan, P.M., Barbour, E.L., and Bohlmann, J.** (2016). Heartwood-specific transcriptome and metabolite signatures of tropical sandalwood (*Santalum album*) reveal the final step of (Z)-santalol fragrance biosynthesis. *Plant J.* **86**:289–299.
- Chaturvedi, R., Giri, M., Chowdhury, Z., Venables, B.J., Mohanty, D., Petros, R.A., and Shah, J.** (2020). CYP720A1 function in roots is required for flowering time and systemic acquired resistance in the foliage of *Arabidopsis*. *J. Exp. Bot.* **71**:6612–6622.
- Cheng, S., Xian, W., Fu, Y., Marin, B., Keller, J., Wu, T., Sun, W., Li, X., Xu, Y., Zhang, Y., et al.** (2019). Genomes of subaerial Zygnematophyceae provide insights into land plant evolution. *Cell* **179**:1057–1067.e14.
- Christ, B., Xu, C., Xu, M., Li, F.S., Wada, N., Mitchell, A.J., Han, X.L., Wen, M.L., Fujita, M., and Weng, J.K.** (2019). Repeated evolution of cytochrome P450-mediated spiroketal steroid biosynthesis in plants. *Nat. Commun.* **10**:3206.
- Christenhusz, M.J.M., and Byng, J.W.** (2016). The number of known plants species in the world and its annual increase. *Phytotaxa* **261**:201–217.
- Clausen, M., Kannangara, R.M., Olsen, C.E., Blomstedt, C.K., Gleadow, R.M., Jørgensen, K., Bak, S., Motawie, M.S., and Møller, B.L.** (2015). The bifurcation of the cyanogenic glucoside and glucosinolate biosynthetic pathways. *Plant J.* **84**:558–573.
- Collu, G., Unver, N., Peltenburg-Looman, A.M.G., van der Heijden, R., Verpoorte, R., and Memelink, J.** (2001). Geraniol 10-hydroxylase, a cytochrome P450 enzyme involved in terpenoid indole alkaloid biosynthesis. *FEBS Lett.* **508**:215–220.
- Cox, C.J., Goffinet, B., Wickett, N.J., Boles, S.B., and Shaw, A.J.** (2010). Moss diversity: a molecular phylogenetic analysis of genera. *Phytotaxa* **9**:175–195.
- Dang, T.T., Franke, J., Tatsis, E., and O'Connor, S.E.** (2017). Dual catalytic activity of a cytochrome P450 controls bifurcation at a metabolic branch point of alkaloid biosynthesis in *Rauwolfia serpentina*. *Angew. Chem. Int. Ed.* **56**:9440–9444.
- De La Pena, R., and Sattely, E.S.** (2020). Rerouting plant terpene biosynthesis enables momilactone pathway elucidation. *Nat. Chem. Biol.* **17**:205–212.
- Delwiche, C.F., and Cooper, E.D.** (2015). The evolutionary origin of a terrestrial flora. *Curr. Biol.* **25**:R899–R910.
- Dimaano, N.G., and Iwakami, S.** (2020). Cytochrome P450-mediated herbicide metabolism in plants: current understanding and prospects. *Pest Manag. Sci.* **77**:22–32.
- Ding, Y., Murphy, K.M., Poretsky, E., Mafu, S., Yang, B., Char, S.N., Christensen, S.A., Saldivar, E., Wu, M., Wang, Q., et al.** (2019). Multiple genes recruited from hormone pathways partition maize diterpenoid defences. *Nat. Plants* **5**:1043–1056.
- Ding, Y., Weckwerth, P.R., Poretsky, E., Murphy, K.M., Sims, J., Saldivar, E., Christensen, S.A., Char, S.N., Yang, B., Tong, A.D., et al.** (2020). Genetic elucidation of interconnected antibiotic pathways mediating maize innate immunity. *Nat. Plants* **6**:1375–1388.
- Dobritsa, A.A., Shrestha, J., Morant, M., Pinot, F., Matsuno, M., Swanson, R., Moller, B.L., and Preuss, D.** (2009). CYP704B1 is a long-chain fatty acid omega-hydroxylase essential for sporopollenin synthesis in pollen of *Arabidopsis*. *Plant Physiol.* **151**:574–589.
- Eberle, D., Ullmann, P., Werck-Reichhart, D., and Petersen, M.** (2009). cDNA cloning and functional characterisation of CYP98A14 and NADPH:cytochrome P450 reductase from *Coleus blumei* involved in rosmarinic acid biosynthesis. *Plant Mol. Biol.* **69**:239–253.
- Ei-Awaad, I., Bocola, M., Beuerle, T., Liu, B., and Beerhues, L.** (2016). Bifunctional CYP81AA proteins catalyse identical hydroxylations but alternative regioselective phenol couplings in plant xanthone biosynthesis. *Nat. Commun.* **7**:11472.
- Eljonaïdi, K., Cankar, K., Comino, C., Moglia, A., Hehn, A., Bourgaud, F., Bouwmeester, H., Menin, B., Klantner, S., and Beekwilder, J.** (2014). Cytochrome P450s from *Cynara cardunculus* L. CYP71AV9 and CYP71BL5, catalyze distinct hydroxylations in the sesquiterpene lactone biosynthesis pathway. *Plant Sci.* **223**:59–68.
- Cooper, E., and Delwiche, C.** (2016). Green algal transcriptomes for phylogenetics and comparative genomics. figshare <https://doi.org/10.6084/m9.figshare.1604778>.
- Farmer, E.E., and Goossens, A.** (2019). Jasmonates: what ALLENE OXIDE SYNTHASE does for plants. *J. Exp. Bot.* **70**:3373–3378.
- Feyereisen, R.** (2011). Arthropod CYPomes illustrate the tempo and mode in P450 evolution. *Biochim. Biophys. Acta* **1814**:19–28.
- Fujiyama, K., Hino, T., Kanadani, M., Watanabe, B., Jae Lee, H., Mizutani, M., and Nagano, S.** (2019). Structural insights into a key step of brassinosteroid biosynthesis and its inhibition. *Nat. Plants* **5**:589–594.
- Fukushima, E.O., Seki, H., Ohyama, K., Ono, E., Umemoto, N., Mizutani, M., Saito, K., and Muranaka, T.** (2011). CYP716A

- subfamily members are multifunctional oxidases in triterpenoid biosynthesis. *Plant Cell Physiol.* **52**:2050–2061.
- Geisler, K., Hughes, R.K., Sainsbury, F., Lomonossoff, G.P., Rejzek, M., Fairhurst, S., Olsen, C.E., Motawia, M.S., Melton, R.E., Hemmings, A.M., et al.** (2013). Biochemical analysis of a multifunctional cytochrome P450 (CYP51) enzyme required for synthesis of antimicrobial triterpenes in plants. *Proc. Natl. Acad. Sci. U S A* **110**:E3360–E3367.
- Geisler, K., Jensen, N.B., Yuen, M.M., Madilao, L., and Bohlmann, J.** (2016). Modularity of conifer diterpene resin acid biosynthesis: P450 enzymes of different CYP720B clades use alternative substrates and converge on the same products. *Plant Physiol.* **171**:152–164.
- Gesell, A., Blaukopf, M., Madilao, L., Yuen, M.M., Withers, S.G., Mattsson, J., Russell, J.H., and Bohlmann, J.** (2015). The gymnosperm cytochrome P450 CYP750B1 catalyzes stereospecific monoterpene hydroxylation of (+)-sabinene in thujone biosynthesis in western redcedar. *Plant Physiol.* **168**:94–106.
- Ginglinger, J.F., Boachon, B., Höfer, R., Paetz, C., Köllner, T.G., Miesch, L., Lugan, R., Baltenweck, R., Mutterer, J., Ullmann, P., et al.** (2013). Gene coexpression analysis reveals complex metabolism of the monoterpene alcohol linalool in *Arabidopsis* flowers. *Plant Cell* **25**:4640–4657.
- Gleadow, R.M., and Møller, B.L.** (2014). Cyanogenic glycosides: synthesis, physiology, and phenotypic plasticity. *Annu. Rev. Plant Biol.* **65**:155–185.
- Gorina, S.S., Toporkova, Y.Y., Mukhtarova, L.S., Smirnova, E.O., Chechetkin, I.R., Khairutdinov, B.I., Gogolev, Y.V., and Grechkin, A.N.** (2016). Oxylipin biosynthesis in spikemoss *Selaginella moellendorffii*: molecular cloning and identification of divinyl ether synthases CYP74M1 and CYP74M3. *Biochim. Biophys. Acta* **1861**:301–309.
- Gotoh, O.** (1992). Substrate recognition sites in cytochrome P450 family 2 (CYP2) proteins inferred from comparative analyses of amino acid and coding nucleotide sequences. *J. Biol. Chem.* **267**:83–90.
- Gu, M., Wang, M., Guo, J., Shi, C., Deng, J., Huang, L., Huang, L., and Chang, Z.** (2019). Crystal structure of CYP76AH1 in 4-PI-bound state from *Salvia miltiorrhiza*. *Biochem. Biophys. Res. Commun.* **511**:813–819.
- Guengerich, F.P., and Munro, A.** (2013). Unusual cytochrome P450 enzymes and reactions. *J. Biol. Chem.* **288**:17065–17073.
- Guiry, M.D.** (2012). How many species of algae are there? *J. Phycol.* **48**:1057–1063.
- Guo, J., Zhou, Y.J., Hillwig, M.L., Shen, Y., Yang, L., Wang, Y., Zhang, X., Liu, W., Peters, R.J., Chen, X., et al.** (2013). CYP76AH1 catalyzes turnover of miltiradiene in tanshinones biosynthesis and enables heterologous production of ferruginol in yeasts. *Proc. Natl. Acad. Sci. U S A* **110**:12108–12113.
- Guo, J., Ma, X., Cai, Y., Ma, Y., Zhan, Z., Zhou, Y.J., Liu, W., Guan, M., Yang, J., Cui, G., et al.** (2016). Cytochrome P450 promiscuity leads to a bifurcating biosynthetic pathway for tanshinones. *New Phytol* **210**:525–534.
- Halkier, B.A., Olsen, C.E., and Møller, B.L.** (1989). The biosynthesis of cyanogenic glucosides in higher plants. *J. Biol. Chem.* **264**:19487–19494.
- Hamberger, B., Ohnishi, T., Hamberger, B., Seguin, A., and Bohlmann, J.** (2011). Evolution of diterpene metabolism: Sitka spruce CYP720B4 catalyzes multiple oxidations in resin acid biosynthesis of conifer defense against insects. *Plant Physiol.* **157**:1677–1695.
- Han, H., Yu, Q., Beffa, R., Gonzalez, S., Maiwald, F., Wang, J., and Powles, S.B.** (2020). Cytochrome P450 CYP81A10v7 in *Lolium rigidum* confers metabolic resistance to herbicides across at least five modes of action. *Plant J.* **105**:79–92.
- Hansen, C.C., Sørensen, M., Veiga, T.A.M., Zibrandtsen, J.F.S., Heskes, A.M., Olsen, C.E., Boughton, B.A., Møller, B.L., and Neilson, E.H.J.** (2018). Reconfigured cyanogenic glucoside biosynthesis in *Eucalyptus cladocalyx* involves a cytochrome P450 CYP706C55. *Plant Physiol.* **178**:1081–1095.
- Hansen, N.L., Miettinen, K., Zhao, Y., Ignea, C., Andreadelli, A., Raadam, M.H., Makris, A.M., Møller, B.L., Staerk, D., Bak, S., et al.** (2020). Integrating pathway elucidation with yeast engineering to produce polypunonic acid the precursor of the anti-obesity agent celastrol. *Microb. Cell Fact.* **19**:15.
- Harholt, J., Moestrup, O., and Ulvskov, P.** (2016). Why plants were terrestrial from the beginning. *Trends Plant Sci.* **21**:96–101.
- Hatlestad, G.J., Sunnadaniya, R.M., Akhavan, N.A., Gonzalez, A., Goldman, I.L., McGrath, J.M., and Lloyd, A.M.** (2012). The beet *R* locus encodes a new cytochrome P450 required for red betalain production. *Nat Genet.* **44**:816–820.
- He, J., Chen, Q., Xin, P., Yuan, J., Ma, Y., Wang, X., Xu, M., Chu, J., Peters, R.J., and Wang, G.** (2019). CYP72A enzymes catalyze 13-hydroxylation of gibberellins. *Nat. Plants* **5**:1057–1065.
- Heitz, T., Widemann, E., Lugan, R., Miesch, L., Ullmann, P., Desaubry, L., Holder, E., Grausem, B., Kandel, S., Miesch, M., et al.** (2012). Cytochromes P450 CYP94C1 and CYP94B3 catalyze two successive oxidation steps of plant hormone jasmonoyl-isoleucine for catabolic turnover. *J. Biol. Chem.* **287**:6296–6306.
- Helliwell, C.A., Chandler, P.M., Poole, A., Dennis, E.S., and Peacock, W.J.** (2001). The CYP88A cytochrome P450, *ent*-kaurenoic acid oxidase, catalyzes three steps of the gibberellin biosynthesis pathway. *Proc. Natl. Acad. Sci. U S A* **98**:2065–2070.
- Helliwell, C.A., Poole, A., Peacock, W.J., and Dennis, E.S.** (1999). *Arabidopsis ent*-kaurene oxidase catalyzes three steps of gibberellin biosynthesis. *Plant Physiol.* **119**:507–510.
- Höfer, R., Dong, L., André, F., Ginglinger, J.F., Lugan, R., Gavira, C., Grec, S., Lang, G., Memelink, J., Van der Krol, S., et al.** (2013). Geraniol hydroxylase and hydroxygeraniol oxidase activities of the CYP76 family of cytochrome P450 enzymes and potential for engineering the early steps of the (seco)iridoid pathway. *Metab Eng* **20**:221–232.
- Holton, T.A., Brugliera, F., Lester, D.R., Tanaka, Y., Hyland, C.D., Menting, J.G.T., Lu, C.-Y., Farcy, E., Stevenson, T.W., and Cornish, E.C.** (1993). Cloning and expression of cytochrome P450 genes controlling flower colour. *Nature* **366**:276–279.
- Hori, K., Maruyama, F., Fujisawa, T., Togashi, T., Yamamoto, N., Seo, M., Sato, S., Yamada, T., Mori, H., Tajima, N., et al.** (2014). *Klebsormidium flaccidum* genome reveals primary factors for plant terrestrial adaptation. *Nat. Commun.* **5**:3978.
- Howe, G.A., Lee, G.I., Itoh, A., Li, L., and DeRocher, A.E.** (2000). Cytochrome P450-dependent metabolism of oxylipins in tomato. Cloning and expression of allene oxide synthase and fatty acid hydroperoxide lyase. *Plant Phys.* **123**:711–724.
- Humphreys, J., Hemm, M.R., and Chapple, C.** (1999). New routes for lignin biosynthesis defined by biochemical characterization of recombinant ferulate 5-hydroxylase, a multifunctional cytochrome P450-dependent monooxygenase. *Proc. Natl. Acad. Sci. U S A* **96**:10045–10050.
- Höfer, R., Boachon, B., Renault, H., Gavira, C., Miesch, L., Iglesias, J., Ginglinger, J.F., Allouche, L., Miesch, M., Grec, S., et al.** (2014). Dual function of the cytochrome P450 CYP76 family from *Arabidopsis thaliana* in the metabolism of monoterpenols and phenylurea herbicides. *Plant Physiol.* **166**:1149–1161.
- Ikezawa, N., Iwasa, K., and Sato, F.** (2008). Molecular cloning and characterization of CYP80G2, a cytochrome P450 that catalyzes an intramolecular C-C phenol coupling of (S)-reticuline in magnoflorine

- biosynthesis, from cultured *Coptis japonica* cells. *J. Biol. Chem.* **283**:8810–8821.
- Ikezawa, N., Tanaka, M., Nagayoshi, M., Shinkyo, R., Sakaki, T., Inouye, K., and Sato, F. (2003). Molecular cloning and characterization of CYP719, a methylenedioxy bridge-forming enzyme that belongs to a novel P450 family, from cultured *Coptis japonica* cells. *J. Biol. Chem.* **278**:38557–38565.
- Ilc, T., Arista, G., Tavares, R., Navrot, N., Duchêne, E., Velt, A., Choulet, F., Paux, E., Fischer, M., Nelson, D.R., et al. (2018). Annotation, classification, genomic organization and expression of the *Vitis vinifera* CYPome. *Plos One* **13**:e0199902.
- Ilc, T., Halter, D., Miesch, L., Lauvoisard, F., Kriegshausen, L., Ilg, A., Baltenweck, R., Hugueney, P., Werck-Reichhart, D., Duchene, E., et al. (2017). A grapevine cytochrome P450 generates the precursor of wine lactone, a key odorant in wine. *New Phytol.* **213**:264–274.
- Ilc, T., Parage, C., Boachon, B., Navrot, N., and Werck-Reichhart, D. (2016). Monoterpenol oxidative metabolism: role in plant adaptation and potential applications. *Front Plant Sci.* **7**:509.
- Irmisch, S., Clavijo McCormick, A., Gunther, J., Schmidt, A., Boeckler, G.A., Gershenzon, J., Unsicker, S.B., and Kollner, T.G. (2014). Herbivore-induced poplar cytochrome P450 enzymes of the CYP71 family convert aldoximes to nitriles which repel a generalist caterpillar. *Plant J.* **80**:1095–1107.
- Irmisch, S., McCormick, A.C., Boeckler, G.A., Schmidt, A., Reichelt, M., Schneider, B., Block, K., Schnitzler, J.P., Gershenzon, J., Unsicker, S.B., et al. (2013a). Two herbivore-induced cytochrome P450 enzymes CYP79D6 and CYP79D7 catalyze the formation of volatile aldoximes involved in poplar defense. *Plant Cell* **25**:4737–4754.
- Irmisch, S., Unsicker, S.B., Gershenzon, J., and Kollner, T.G. (2013b). Identification and characterization of CYP79D6v4, a cytochrome P450 enzyme producing aldoximes in black poplar (*Populus nigra*). *Plant Signal Behav.* **8**:e27640.
- Irmiler, S., Schröder, G., St-Pierre, B., Crouch, N.P., Hotze, M., Schmidt, J., Strack, D., Matern, U., and Schröder, J. (2000). Indole alkaloid biosynthesis in *Catharanthus roseus*: new enzyme activities and identification of cytochrome P450 CYP72A1 as secologanin synthase. *Plant J.* **24**:797–804.
- Jiao, C., Sorensen, I., Sun, X., Sun, H., Behar, H., Alseekh, S., Philippe, G., Palacio Lopez, K., Sun, L., Reed, R., et al. (2020). The *Penium margaritaceum* genome: hallmarks of the origins of land plants. *Cell* **181**:1097–1111.e12.
- Kahn, R.A., Bak, S., Svendsen, I., Halkier, B.A., and Møller, B.L. (1997). Isolation and reconstitution of cytochrome P450ox and in vitro reconstitution of the entire biosynthetic pathway of the cyanogenic glucoside dhurrin from sorghum. *Plant Physiol.* **115**:1661–1670.
- Kahn, R.A., Fahrendorf, T., Halkier, B.A., and Møller, B.L. (1999). Substrate specificity of the cytochrome P450 enzymes CYP79A1 and CYP71E1 involved in the biosynthesis of the cyanogenic glucoside dhurrin in *Sorghum bicolor* (L.) Moench. *Arch. Biochem. Biophys.* **363**:9–18.
- Keeling, P.J. (2010). The endosymbiotic origin, diversification and fate of plastids 365:729–748.
- Kellner, F., Geu-Flores, F., Sherden, N.H., Brown, S., Foureau, E., Courdavault, V., and O'Connor, S. (2015). Discovery of a P450-catalyzed step in vindoline biosynthesis: a link between the aspidosperma and eburnamine alkaloids. *Chem. Commun.* **51**:7626–7628.
- Kilgore, M.B., Augustin, M.M., May, G.D., Cow, J.A., and Kutchan, T.M. (2016). CYP96T1 of *Narcissus* sp. aff. *Pseudonarcissus* catalyzes formation of the para-para' C-C phenol couple in the Amaryllidaceae alkaloids. *Front Plant Sci.* **7**:225.
- Kim, T.-K., Hwang, J.-Y., Kim, Y.-S., Joo, S.-H., Chang, S.C., Lee, J.S., Takatsuto, S., and Kim, S.-K. (2005). *Arabidopsis* CYP85A2, a cytochrome P450, mediates the Baeyer-Villiger oxidation of castasterone to brassinolide in brassinosteroid biosynthesis. *Plant Cell* **17**:2397–2412.
- Kim, J., Smith, J.J., Tian, L., and Dellapenna, D. (2009). The evolution and function of carotenoid hydroxylases in *Arabidopsis*. *Plant Cell Physiol.* **50**:463–479.
- Kitaoka, N., Zhang, J., Oyabenro, R.K., Brown, B., Wu, Y., Yang, B., Li, Z., and Peters, R.J. (2021). Interdependent evolution of biosynthetic gene clusters for momilactone production in rice. *Plant Cell* **33**:290–305.
- Klein, A.P., and Sattely, E.S. (2015). Two cytochrome P450 catalyze S-heterocyclizations in cabbage phytoalexin biosynthesis. *Nat. Chem. Biol.* **11**:837–839.
- Knudsen, C., Gallage, N.J., Hansen, C.C., Møller, B.L., and Laursen, T. (2018). Dynamic metabolic solutions to the sessile life style of plants. *Nat. Prod. Rep.* **35**:1140–1155.
- Koch, B.M., Sibbesen, O., Halkier, B.A., Svendsen, I., and Møller, B.L. (1995). The primary sequence of cytochrome P450tyr, the multifunctional N-hydroxylase catalyzing the conversion of L-tyrosine to p-hydroxyphenylacetaldehyde oxime in the biosynthesis of the cyanogenic glucoside dhurrin in *Sorghum bicolor* (L.) Moench. *Arch. Biochem. Biophys.* **323**:177–186.
- Koeduka, T., Ishizaki, K., Mwenda, C.M., Hori, K., Sasaki-Sekimoto, Y., Ohta, H., Kohchi, T., and Matsui, K. (2015). Biochemical characterization of allene oxide synthases from the liverwort *Marchantia polymorpha* and green microalgae *Klebsormidium flaccidum* provides insight into the evolutionary divergence of the plant CYP74 family. *Planta* **242**:1175–1186.
- Krieger, C., Roselli, S., Kellner-Thielmann, S., Galati, G., Schneider, B., Grosjean, J., Olry, A., Ritchie, D., Matern, U., Bourgaud, F., et al. (2018). The CYP71AZ P450 subfamily: a driving factor for the diversification of coumarin biosynthesis in apiaceous plants. *Front Plant Sci.* **9**:820.
- Kriegshausen, L., Knosp, S., Grienberger, E., Tatsumi, K., Gütle, D.D., Sørensen, I., Herrgott, L., Zumsteg, J., Rose, J.K.C., Reski, R., et al. (2021). Function of the HYDROXYCINNAMOYL-CoA:SHIKIMATE HYDROXYCINNAMOYL TRANSFERASE is evolutionarily conserved in embryophytes. *Plant Cell* <https://doi.org/10.1093/plcell/koab044>.
- Kunii, M., Kitahama, Y., Fukushima, E.O., Seki, H., Muranaka, T., Yoshida, Y., and Aoyama, Y. (2012). β -Amyrin oxidation by oat CYP51H10 expressed heterologously in yeast cells: the first example of CYP51-dependent metabolism other than the 14-demethylation of sterol precursors. *Biol. Pharm. Bull.* **35**:801–804.
- Le Bouquin, R., Pinot, F., Benveniste, I., Salaün, J.-P., and Durst, F. (1999). Cloning and functional characterization of CYP94A2, a medium chain fatty acid hydroxylase from *Vicia sativa*. *Biochem. Biophys. Res. Commun.* **261**:156–162.
- Lee, D.S., Nioche, P., Hamberg, M., and Raman, C.S. (2008). Structural insights into the evolutionary paths of oxylipin biosynthetic enzymes. *Nature* **455**:363–368.
- Lee, S., Badiyan, S., Bevan, D.R., Herde, M., Gatz, C., and Tholl, D. (2010). Herbivore-induced and floral homoterpene volatiles are biosynthesized by a single P450 enzyme (CYP82G1) in *Arabidopsis*. *Proc. Natl. Acad. Sci. U S A* **107**:21205–21210.
- Levsh, O., Pluskal, T., Carballo, V., Mitchell, A.J., and Weng, J.K. (2019). Independent evolution of rosmarinic acid biosynthesis in two sister families under the Lamiids clade of flowering plants. *J. Biol. Chem.* **294**:15193–15205.

- Li, J., Halitschke, R., Li, D., Paetz, C., Su, H., Heiling, S., Xu, S., and Baldwin, I.T. (2021). Controlled hydroxylations of diterpenoids allow for plant chemical defense without autotoxicity. *Science* **371**:255–260.
- Li, L., Chang, Z., Pan, Z., Fu, Z.-Q., and Wang, X. (2008). Modes of heme binding and substrate access for cytochrome P450 CYP74A revealed by crystal structures of allene oxide synthase 105:13883–13888.
- Li, L., Wang, S., Wang, H., Sahu, S.K., Marin, B., Li, H., Xu, Y., Liang, H., Li, Z., Cheng, S., et al. (2020). The genome of *Prasinoderma coloniale* unveils the existence of a third phylum within green plants. *Nat. Ecol. Evol.* **4**:1220–1231.
- Li, R., Reed, D.W., Liu, E., Nowak, J., Pelcher, L.E., Page, J.E., and Covello, P.S. (2006). Functional genomic analysis of alkaloid biosynthesis in *Hyoscyamus niger* reveals a cytochrome P450 involved in littorine rearrangement. *Chem. Biol.* **13**:513–520.
- Liang, M.H., Xie, H., Chen, H.H., Liang, Z.C., and Jiang, J.G. (2020). Functional identification of two types of carotene hydroxylases from the green alga *Dunaliella bardawil* rich in lutein. *ACS Synth. Biol.* **9**:1246–1253.
- Limonos-Mendez, M., Dugrand-Judek, A., Villard, C., Coqueret, V., Froelicher, Y., Bourgaud, F., Olry, A., and Hehn, A. (2020). Convergent evolution leading to the appearance of furanocoumarins in citrus plants. *Plant Sci.* **292**:110392.
- Liu, Q., Khakimov, B., Cardenas, P.D., Cozzi, F., Olsen, C.E., Jensen, K.R., Hauser, T.P., and Bak, S. (2019). The cytochrome P450 CYP72A552 is key to production of hederagenin-based saponins that mediate plant defense against herbivores. *New Phytol.* **222**:1599–1609.
- Liu, X., Cheng, J., Zhang, G., Ding, W., Duan, L., Yang, J., Kui, L., Cheng, X., Ruan, J., Fan, W., et al. (2018). Engineering yeast for the production of breviscapine by genomic analysis and synthetic biology approaches. *Nat. Commun.* **9**:448.
- Liu, Z., Boachon, B., Lugan, R., Tavares, R., Erhardt, M., Mutterer, J., Demais, V., Pateyron, S., Brunaud, V., Ohnishi, T., et al. (2015). A conserved cytochrome P450 evolved in seed plants regulates flower maturation. *Mol. Plant* **8**:1751–1765.
- Liu, Z., Tavares, R., Forsythe, E.S., Andre, F., Lugan, R., Jonasson, G., Boutet-Mercey, S., Tohge, T., Beilstein, M.A., Werck-Reichhart, D., et al. (2016). Evolutionary interplay between sister cytochrome P450 genes shapes plasticity in plant metabolism. *Nat. Commun.* **7**:13026.
- Luck, K., Jirschtzka, J., Irmisch, S., Huber, M., Gershenzon, J., and Köllner, T.G. (2016). CYP79D enzymes contribute to jasmonic acid-induced formation of aldoximes and other nitrogenous volatiles in two *Erythroxylum* species. *BMC Plant Biol.* **16**:215.
- Lui, A.C.W., Lam, P.Y., Kwun Ho, C., Wang, L., Tobimatsu, Y., and Lo, C. (2020). Convergent recruitment of 5'-hydroxylase activities by CYP75B flavonoid B-ring hydroxylases for tricin biosynthesis in *Medicago* legumes. *New Phytol.* **228**:269–284.
- Luo, P., Wang, Y.-H., Wang, G.-D., Essenberg, M., and Chen, X.-Y. (2001). Molecular cloning and functional identification of (+)- δ -canadiene-8-hydroxylase, a cytochrome P450 mono-oxygenase (CYP706B1) of cotton sesquiterpene biosynthesis. *Plant J.* **28**:95–104.
- Maeda, S., Dubouzet, J.G., Kondou, Y., Jikumaru, Y., Seo, S., Oda, K., Matsui, M., Hirochika, H., and Mori, M. (2019). The rice CYP78A gene BSR2 confers resistance to *Rhizoctonia solani* and affects seed size and growth in *Arabidopsis* and rice. *Sci. Rep.* **9**:587.
- Mafu, S., Ding, Y., Murphy, K.M., Yaacoobi, O., Addison, J.B., Wang, Q., Shen, Z., Briggs, S.P., Bohlmann, J., Castro-Falcon, G., et al. (2018). Discovery, biosynthesis and stress-related accumulation of dolabradiene-derived defenses in maize. *Plant Physiol.* **176**:2677–2690.
- Mafu, S., Jia, M., Zi, J., Morrone, D., Wu, Y., Xu, M., Hillwig, M.L., and Peters, R.J. (2016). Probing the promiscuity of ent-kaurene oxidases via combinatorial biosynthesis. *Proc. Natl. Acad. Sci. U S A* **113**:2526–2531.
- Mahesh, V., Million-Rousseau, R., Ullmann, P., Chabrilange, N., Bustamante, J., Mondolot, L., Morant, M., Noiro, M., Hamon, S., de Kochko, A., et al. (2007). Functional characterization of two *p*-coumaroyl ester 3'-hydroxylase genes from coffee tree: evidence of a candidate for chlorogenic acid biosynthesis. *Plant Mol. Biol.* **64**:145–159.
- Mao, L., Kawaide, H., Higuchi, T., Chen, M., Miyamoto, K., Hirata, Y., Kimura, H., Miyazaki, S., Teruya, M., Fujiwara, K., et al. (2020). Genomic evidence for convergent evolution of gene clusters for momilactone biosynthesis in land plants. *Proc. Natl. Acad. Sci. U S A* **117**:12472–12480.
- Matsuno, M., Compagnon, V., Schoch, G.A., Schmitt, M., Debayle, D., Bassard, J.-E., Pollet, B., Hehn, A., Heintz, D., Ullmann, P., et al. (2009). Evolution of a novel phenolic pathway for pollen development. *Science* **325**:1688–1692.
- Matsuno, M., Nagatsu, A., Ogihara, Y., Ellis, B.E., and Mizukami, H. (2002). CYP98A6 from *Lithospermum erythrorhizon* encodes 4-coumaroyl-4'-hydroxyphenyllactic acid 3-hydroxylase involved in rosmarinic acid biosynthesis. *FEBS Lett.* **514**:219–224.
- Meyer, K., Shirley, A.M., Cusumano, J.C., Bell-Lelong, D.A., and Chapple, C. (1998). Lignin monomer composition is determined by the expression of a cytochrome P450-dependent monooxygenase in *Arabidopsis*. *Proc. Natl. Acad. Sci. U S A* **95**:6619–6623.
- Miettinen, K., Dong, L., Navrot, N., Schneider, T., Burlat, V., Pollier, J., Woittiez, L., van der Krol, S., Lugan, R., Ilc, T., et al. (2014). The seco-iridoid pathway from *Catharanthus roseus*. *Nat. Commun.* **5**:3606.
- Miettinen, K., Pollier, J., Buyst, D., Arendt, P., Csuk, R., Sommerwerk, S., Moses, T., Mertens, J., Sonawane, P.D., Pauwels, L., et al. (2017). The ancient CYP716 family is a major contributor to the diversification of eudicot triterpenoid biosynthesis. *Nat. Commun.* **8**:14153.
- Miyazaki, S., Katsumata, T., Natsume, M., and Kawaide, H. (2011). The CYP701B1 of *Physcomitrella patens* is an ent-kaurene oxidase that resists inhibition by uniconazole-P. *FEBS Lett.* **585**:1879–1883.
- Mizutani, M., and Sato, F. (2011). Unusual P450 reactions in plant secondary metabolism. *Arch. Biochem. Biophys.* **507**:194–203.
- Morant, M., Schoch, G.A., Ullmann, P., Ertunc, T., Little, D., Olsen, C.E., Petersen, M., Negrel, J., and Werck-Reichhart, D. (2007). Catalytic activity, duplication and evolution of the CYP98 cytochrome P450 family in wheat. *Plant Mol. Biol.* **63**:1–19.
- Moreno, J.C., Mi, J., Alagoz, Y., and Al-Babili, S. (2021). Plant apocarotenoids: from retrograde signaling to interspecific communication. *Plant J.* **105**:351–375.
- Mori, N., Sado, A., Xie, X., Yoneyama, K., Asami, K., Seto, Y., Nomura, T., Yamaguchi, S., Yoneyama, K., and Akiyama, K. (2020). Chemical identification of 18-hydroxycaractonoic acid as an *Lj*MAX1 product and in planta conversion of its methyl ester to canonical and non-canonical strigolactones in *Lotus japonicus*. *Phytochemistry* **174**:112349.
- Morikawa, T., Mizutani, M., Aoki, N., Watanabe, B., Saga, H., Saito, S., Oikawa, A., Suzuki, H., Sakurai, N., Shibata, D., et al. (2006a). Cytochrome P450 CYP710A encodes the sterol C-22 desaturase in *Arabidopsis* and tomato. *Plant Cell* **18**:1008–1022.
- Morikawa, T., Mizutani, M., and Ohta, D. (2006b). Cytochrome P450 subfamily CYP710A genes encode sterol C-22 desaturase in plants. *Biochem. Soc. Trans.* **34**:1202–1205.
- Morrone, D., Chen, X., Coates, R.M., and Peters, R.J. (2010). Characterization of the kaurene oxidase CYP701A3, a multifunctional cytochrome P450 from gibberellin biosynthesis. *Biochem. J.* **431**:337–344.

- Møller, B.L. (2017). Solar-driven synthesis of bioactive natural products. *Chem. Eng. Prog.* **113**:46–54.
- Nasomjai, P., Reed, D.W., Tozer, D.J., Peach, M.J.G., Slawin, A.M.Z., Covello, P.S., and O'Hagan, D. (2009). Mechanistic insights into the cytochrome P450-mediated oxidation and rearrangement of litorine in tropane alkaloid biosynthesis. *ChemBioChem* **10**:2382–2393.
- Nelson, D. (2009). The cytochrome P450 homepage. *Hum. Genomics* **4**:59–65.
- Nelson, D., and Werck-Reichhart, D. (2011). A P450-centric view of plant evolution. *Plant J.* **66**:194–211.
- Nelson, D.R. (2006). Plant cytochrome P450s from moss to poplar. *Phytochem. Rev.* **5**:193–204.
- Nelson, D.R. (2018). Cytochrome P450 diversity in the tree of life. *Biochim. Biophys. Acta Proteins Proteom.* **1866**:141–154.
- Nett, R.S., Lau, W., and Sattely, E.S. (2020). Discovery and engineering of colchicine alkaloid biosynthesis. *Nature* **584**:148–153.
- Nguyen, D.T., Gopfert, J.C., Ikezawa, N., Macnevin, G., Kathiresan, M., Conrad, J., Spring, O., and Ro, D.K. (2010). Biochemical conservation and evolution of germacrene A oxidase in Asteraceae. *J. Biol. Chem.* **285**:16588–16598.
- Nguyen, T.D., Kwon, M., Kim, S.U., Fischer, C., and Ro, D.K. (2019). Catalytic plasticity of germacrene A oxidase underlies sesquiterpene lactone diversification. *Plant Physiol.* **181**:945–960.
- Nishiyama, T., Sakayama, H., de Vries, J., Buschmann, H., Saint-Marcoux, D., Ullrich, K.K., Haas, F.B., Vanderstraeten, L., Becker, D., Lang, D., et al. (2018). The Chara genome: secondary complexity and implications for plant terrestrialization. *Cell* **174**:448–464.e24.
- Niu, G., Guo, Q., Wang, J., Zhao, S., He, Y., and Liu, L. (2020). Structural basis for plant lutein biosynthesis from alpha-carotene. *Proc. Natl. Acad. Sci. U S A* **117**:14150–14157.
- One Thousand Plant Transcriptomes, I. (2019). One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* **574**:679–685.
- Pateraki, I., Andersen-Ranberg, J., Jensen, N.B., Wubshet, S.G., Heskes, A.M., Forman, V., Hallstrom, B., Hamberger, B., Motawia, M.S., Olsen, C.E., et al. (2017). Total biosynthesis of the cyclic AMP booster forskolin from *Coleus forskohlii*. *eLife* **6**:e23001.
- Pathak, M.A., Daniels, F., Jr., and Fitzpatrick, T.B. (1962). The presently known distribution of furocoumarins (psoralens) in plants. *J. Invest. Dermatol.* **39**:225–239.
- Petersen, M., Abdullah, Y., Benner, J., Eberle, D., Gehlen, K., Hücherig, S., Janiak, V., Kim, K.H., Sander, M., Weitzel, C., et al. (2009). Evolution of rosmarinic acid biosynthesis. *Phytochemistry* **70**:1663–1679.
- Polturak, G., Breitel, D., Grossman, N., Sarrion-Perdigones, A., Weithorn, E., Pliner, M., Orzaez, D., Granell, A., Rogachev, I., and Aharoni, A. (2016). Elucidation of the first committed step in betalain biosynthesis enables the heterologous engineering of betalain pigments in plants. *New Phytol* **210**:269–283.
- Poretska, O., Yang, S., Pitorre, D., Poppenberger, B., and Sieberer, T. (2020). AMP1 and CYP78A5/7 act through a common pathway to govern cell fate maintenance in *Arabidopsis thaliana*. *Plos Genet.* **16**:e1009043.
- PPG, I. (2016). A community-derived classification for extant lycophytes and ferns. *J. Syst. Evol.* **54**:563–603.
- Prasad, K.V.S.K., Song, B.-H., Olson-Manning, C., Anderson, J.T., Lee, C.-R., Schranz, E., Windsor, A.J., Clauss, M.J., Manzaneda, A.J., Naqvi, I., et al. (2012). A gain-of-function polymorphism controlling complex traits and fitness in nature. *Science* **337**:1081–1084.
- Qi, X., Bakht, S., Qin, B., Leggett, M., Hemmings, A., Mellon, F., Eagles, J., Werck-Reichhart, D., Schaller, H., Lesot, A., et al. (2006). A different function for a member of an ancient and highly conserved cytochrome P450 family: from essential sterols to plant defense. *Proc. Natl. Acad. Sci. U S A* **103**:18848–18853.
- Rajniak, J., Giehl, R.F.H., Chang, E., Murgia, I., von Wiren, N., and Sattely, E.S. (2018). Biosynthesis of redox-active metabolites in response to iron deficiency in plants. *Nat. Chem. Biol.* **14**:442–450.
- Ralston, L., Kwon, S.T., Schoenbeck, M., Ralston, J., Schenk, D.J., Coates, R.M., and Chappell, J. (2001). Cloning, heterologous expression, and functional characterization of 5-epi-aristolochene-1,3-dihydroxylase from tobacco (*Nicotiana tabacum*). *Arch. Biochem. Biophys.* **393**:222–235.
- Renault, H., Alber, A., Horst, N.A., Basilio Lopes, A., Fich, E.A., Kriegshauser, L., Wiedemann, G., Ullmann, P., Herrgott, L., Erhardt, M., et al. (2017a). A phenol-enriched cuticle is ancestral to lignin evolution in land plants. *Nat. Commun.* **8**:14713.
- Renault, H., De Marothy, M., Jonasson, G., Lara, P., Nelson, D.R., Nilsson, I., Andre, F., von Heijne, G., and Werck-Reichhart, D. (2017b). Gene duplication leads to altered membrane topology of a cytochrome P450 enzyme in seed plants. *Mol. Biol. Evol.* **34**:2041–2056.
- Rensing, S.A. (2020). How plants conquered land. *Cell* **181**:964–966.
- Rensing, S.A., Goffinet, B., Meyberg, R., Wu, S.Z., and Bezanilla, M. (2020). The moss *Physcomitrium (Physcomitrella) patens*: a model organism for non-seed plants. *Plant Cell* **32**:1361–1376.
- Ro, D.-K., Arimura, G.-I., Lau, S.Y.W., Piers, E., and Bohlmann, J. (2005). Loblolly pine abietadienol/abietadienal oxidase PtAO (CYP720B1) is a multifunctional, multisubstrate cytochrome P450 monooxygenase. *Proc. Natl. Acad. Sci. U S A* **102**:8060–8065.
- Ro, D.K., Paradise, E.M., Ouellet, M., Fisher, K.J., Newman, K.L., Ndungu, J.M., Ho, K.A., Eachus, R.A., Ham, T.S., Kirby, J., et al. (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* **440**:940–943.
- Rodriguez-Lopez, C.E., Hong, B., Paetz, C., Nakamura, Y., Koudounas, K., Passeri, V., Baldoni, L., Alagna, F., Calderini, O., and O'Connor, S.E. (2020). Two bi-functional cytochrome P450 CYP72 enzymes from olive (*Olea europaea*) catalyze the oxidative C-C bond cleavage in the biosynthesis of secoxy-iridoids - flavor and quality determinants in olive oil. *New Phytol.* **229**:2288–2301.
- Salim, V., Yu, F., Altarejos, J., and De Luca, V. (2013). Virus-induced gene silencing identifies *Catharanthus roseus* 7-deoxyloganic acid-7-hydroxylase, a step in iridoid and monoterpene indole alkaloid biosynthesis. *Plant J.* **76**:754–765.
- Scheler, U., Brandt, W., Porzel, A., Rothe, K., Manzano, D., Bozic, D., Papaefthimiou, D., Balcke, G.U., Henning, A., Lohse, S., et al. (2016). Elucidation of the biosynthesis of carosic acid and its reconstitution in yeast. *Nat. Commun.* **7**:12942.
- Scholz, J., Brodhun, F., Hornung, E., Herrfurth, C., Stumpe, M., Beike, A.K., Faltin, B., Frank, W., Reski, R., and Feussner, I. (2012). Biosynthesis of allene oxides in *Physcomitrella patens*. *BMC Plant Biol.* **12**:228.
- Seitz, C., Ameres, S., and Forkmann, G. (2007). Identification of the molecular basis for the functional difference between flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase. *FEBS Lett.* **581**:3429–3434.
- Seitz, C., Ameres, S., Schlangen, K., Forkmann, G., and Halbwirth, H. (2015). Multiple evolution of flavonoid 3',5'-hydroxylase. *Planta* **242**:561–573.
- Seitz, C., Eder, C., Deiml, B., Kellner, S., Martens, S., and Forkmann, G. (2006). Cloning, functional identification and sequence analysis of flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase cDNAs

- reveals independent evolution of flavonoid 3',5'-hydroxylase in the Asteraceae family. *Plant Mol. Biol.* **61**:365–381.
- Shimura, K., Okada, A., Okada, K., Jikumaru, Y., Ko, K.W., Toyomasu, T., Sassa, T., Hasegawa, M., Kodama, O., Shibuya, N., et al.** (2007). Identification of a biosynthetic gene cluster in rice for momilactones. *J. Biol. Chem.* **282**:34013–34018.
- Sibbesen, O., Koch, B., Halkier, B.A., and Møller, B.L.** (1995). Cytochrome P-450TYR is a multifunctional heme-Thiolate enzyme catalyzing the conversion of L-tyrosine to p-hydroxyphenylacetaldehyde oxime in the biosynthesis of the cyanogenic glucoside dhurrin in *Sorghum bicolor* (L.) Moench. *J. Biol. Chem.* **270**:3506–3511.
- Sintupachee, S., Ngamrojanavanich, N., Sitthithaworn, W., and De-Eknamkul, W.** (2014). Molecular cloning, bacterial expression and functional characterisation of cytochrome P450 monooxygenase, CYP97C27, and NADPH-cytochrome P450 reductase, CPR I, from *Croton stellatopilosus* Ohba. *Plant Sci.* **229**:131–141.
- Sircar, D., Gaid, M.M., Chizzali, C., Reckwell, D., Kaufholdt, D., Beuerle, T., Broggin, G.A., Flachowsky, H., Liu, B., Hansch, R., et al.** (2015). Biphenyl 4-hydroxylases involved in aucuparin biosynthesis in rowan and apple are cytochrome P450 736A proteins. *Plant Physiol.* **168**:428–442.
- Sunnadeniya, R., Bean, A., Brown, M., Akhavan, N., Hatlestad, G., Gonzalez, A., Symonds, V.V., Lloyd, A., et al.** (2016). Tyrosine Hydroxylation in Betalain Pigment Biosynthesis Is Performed by Cytochrome P450 Enzymes in Beets (*Beta vulgaris*). *PLoS One* **11**:e0149417.
- Swaminathan, S., Morrone, D., Wang, Q., Fulton, B., and Peters, R.J.** (2009). CYP76M7 Is an ent-Cassadiene C11 α -hydroxylase defining a second multifunctional diterpenoid biosynthetic gene cluster in rice. *Plant Cell* **21**:3315–3325.
- Takos, A.M., Knudsen, C., Lai, D., Kannangara, R., Mikkelsen, L., Motawia, M.S., Olsen, C.E., Sato, S., Tabata, S., Jorgensen, K., et al.** (2011). Genomic clustering of cyanogenic glucoside biosynthetic genes aids their identification in *Lotus japonicus* and suggests the repeated evolution of this chemical defence pathway. *Plant J.* **68**:273–286.
- Teoh, K.H., Polichuk, D.R., Reed, D.W., Nowak, G., and Covello, P.S.** (2006). *Artemisia annua* L. (Asteraceae) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. *FEBS Lett.* **580**:1411–1416.
- Thodberg, S., Del Cueto, J., Mazzeo, R., Pavan, S., Lotti, C., Dicenta, F., Jakobsen Neilson, E.H., Møller, B.L., and Sanchez-Perez, R.** (2018). Elucidation of the amygdalin pathway reveals the metabolic basis of bitter and sweet almonds (*Prunus dulcis*). *Plant Physiol.* **178**:1096–1111.
- Thodberg, S., Hansen, C.C., Takos, A.M., Pičmanová, M., Møller, B.L., Nelson, D.R., and Neilson, E.H.J.** (2021). The fern CYPome: fern-specific cytochrome P450 family involved in convergent evolution of chemical defense. *bioRxiv* <https://doi.org/10.1101/2021.03.23.436569>.
- Thodberg, S., Sørensen, M., Bellucci, M., Crocoll, C., Bendtsen, A.K., Nelson, D.R., Motawia, M.S., Møller, B.L., and Neilson, E.H.J.** (2020). A flavin-dependent monooxygenase catalyzes the initial step in cyanogenic glycoside synthesis in ferns. *Commun. Biol.* **3**:507.
- Thyssen, G.N., Naoumkina, M., McCarty, J.C., Jenkins, J.N., Florane, C., Li, P., and Fang, D.D.** (2018). The P450 gene CYP749A16 is required for tolerance to the sulfonylurea herbicide trifloxysulfuron sodium in cotton (*Gossypium hirsutum* L.). *BMC Plant Biol.* **18**:186.
- Tijet, N., Helvig, C., Pinot, F., Le Bouquin, R., Lesot, A., Durst, F., Salaün, J.-P., and Benveniste, I.** (1998). Functional expression in yeast and characterization of a clofibrate-inducible plant cytochrome P-450 (CYP94A1) involved in cutin monomers synthesis. *Biochem. J.* **332**:582–589.
- Timoneda, A., Feng, T., Sheehan, H., Walker-Hale, N., Pucker, B., Lopez-Nieves, S., Guo, R., and Brockington, S.** (2019). The evolution of betalain biosynthesis in Caryophyllales. *New Phytol.* **224**:71–85.
- Toporkova, Y.Y., Ermilova, V.S., Gorina, S.S., Mukhtarova, L.S., Osipova, E.V., Gogolev, Y.V., and Grechkin, A.N.** (2013). Structure-function relationship in the CYP74 family: conversion of divinyl ether synthases into allene oxide synthases by site-directed mutagenesis. *FEBS Lett.* **587**:2552–2558.
- Toporkova, Y.Y., Gogolev, Y.V., Mukhtarova, L.S., and Grechkin, A.N.** (2008). Determinants governing the CYP74 catalysis: conversion of allene oxide synthase into hydroperoxide lyase by site-directed mutagenesis. *FEBS Lett.* **582**:3423–3428.
- Toporkova, Y.Y., Smirnova, E.O., Mukhtarova, L.S., Gorina, S.S., and Grechkin, A.N.** (2020). Catalysis by allene oxide synthases (CYP74A and CYP74C): alterations by the Phe/Leu mutation at the SRS-1 region. *Phytochemistry* **169**:112152.
- Umamoto, N., Nakayasu, M., Ohyama, K., Yotsu-Yamashita, M., Mizutani, M., Seki, H., Saito, K., and Muranaka, T.** (2016). Two cytochrome P450 monooxygenases catalyze early hydroxylation steps in the potato steroid glycoalkaloid biosynthetic pathway. *Plant Physiol.* **171**:2458–2467.
- Villarreal, J.C., Cargill, D.C., Hagborg, A., Soderstrom, L., and Renzaglia, K.S.** (2010). A synthesis of hornwort diversity: patterns, causes and future work. *Phytotaxa* **9**:150–166.
- Von Konrat, M., Soderstrom, L., Renner, M.A.M., Hagborg, A., Briscoe, L., and Engel, J.J.** (2010). Early Land Plants Today (ELPT): how many liverwort species are there? *Phytotaxa* **9**:22–40.
- Wakabayashi, T., Hamana, M., Mori, A., Akiyama, R., Ueno, K., Osakabe, K., Osakabe, Y., Suzuki, H., Takikawa, H., Mizutani, M., et al.** (2019). Direct conversion of carlactonoic acid to orobanchol by cytochrome P450 CYP722C in strigolactone biosynthesis. *Sci. Adv.* **5**:eaax9067.
- Wakabayashi, T., Shida, K., Kitano, Y., Takikawa, H., Mizutani, M., and Sugimoto, Y.** (2020). CYP722C from *Gossypium arboreum* catalyzes the conversion of carlactonoic acid to 5-deoxystrigol. *Planta* **251**:97.
- Wang, G., Xu, J., Li, L., Guo, Z., Si, Q., Zhu, G., Wang, X., and Guo, W.** (2020a). GbCYP86A1-1 from *Gossypium barbadense* positively regulates defence against *Verticillium dahliae* by cell wall modification and activation of immune pathways. *Plant Biotechnol. J.* **18**:222–238.
- Wang, J., Liu, Y., Cai, Y., Zhang, F., Xia, G., and Xiang, F.** (2010). Cloning and functional analysis of geraniol 10-hydroxylase, a cytochrome P450 from *Swertia mussootii* Franch. *Biosci. Biotechnol. Biochem.* **74**:1583–1590.
- Wang, Q., Hillwig, M.L., Okada, K., Yamazaki, K., Wu, Y., Swaminathan, S., Yamane, H., and Peters, R.J.** (2012a). Characterization of CYP76M5-8 indicates metabolic plasticity within a plant biosynthetic gene cluster. *J. Biol. Chem.* **287**:6159–6168.
- Wang, Q., Hillwig, M.L., and Peters, R.J.** (2011). CYP99A3: functional identification of a diterpene oxidase from the momilactone biosynthetic gene cluster in rice. *Plant J.* **65**:87–95.
- Wang, Q., Hillwig, M.L., Wu, Y., and Peters, R.J.** (2012b). CYP701A8: a rice ent-kaurene oxidase paralog diverted to more specialized diterpenoid metabolism. *Plant Physiol.* **158**:1418–1425.
- Wang, S., Li, L., Li, H., Sahu, S.K., Wang, H., Xu, Y., Xian, W., Song, B., Liang, H., Cheng, S., et al.** (2020b). Genomes of early-diverging streptophyte algae shed light on plant terrestrialization. *Nat. Plants* **6**:95–106.

- Wasternack, C., and Feussner, I. (2018). The oxylipin pathways: biochemistry and function. *Annu. Rev. Plant Biol.* **69**:363–386.
- Weng, J.-K., Li, Y., Mo, H., and Chapple, C. (2012). Assembly of an evolutionary new pathway for α -pyrone biosynthesis in *Arabidopsis*. *Science* **337**:960–964.
- Weng, J.K., Akiyama, T., Bonawitz, N.D., Li, X., Ralph, J., and Chapple, C. (2010). Convergent evolution of syringyl lignin biosynthesis via distinct pathways in the lycophyte *Selaginella* and flowering plants. *Plant Cell* **22**:1033–1045.
- Weng, J.K., Li, X., Stout, J., and Chapple, C. (2008). Independent origins of syringyl lignin in vascular plants. *Proc. Natl. Acad. Sci. U S A* **105**:7887–7892.
- Widemann, E., Grausem, B., Renault, H., Pineau, E., Heinrich, C., Lugan, R., Ullmann, P., Miesch, L., Aubert, Y., Miesch, M., et al. (2015). Sequential oxidation of jasmonoyl-phenylalanine and jasmonoyl-isoleucine by multiple cytochrome P450 of the CYP94 family through newly identified aldehyde intermediates. *Phytochemistry* **117**:388–399.
- Wu, Y., Hillwig, M.L., Wang, Q., and Peters, R.J. (2011). Parsing a multifunctional biosynthetic gene cluster from rice: biochemical characterization of CYP71Z6 & 7. *FEBS Lett.* **585**:3446–3451.
- Wu, Y., Wang, Q., Hillwig, M.L., and Peters, R.J. (2013). Picking sides: distinct roles for CYP76M6 and CYP76M8 in rice oryzalexin biosynthesis. *Biochem. J.* **454**:209–216.
- Xiao, F., Goodwin, S.M., Xiao, Y., Sun, Z., Baker, D., Tang, X., Jenks, M.A., and Zhou, J.M. (2004). *Arabidopsis* CYP86A2 represses *Pseudomonas syringae* type III genes and is required for cuticle development. *EMBO J.* **23**:2903–2913.
- Xu, J., Ding, Z., Vizcay-Barrena, G., Shi, J., Liang, W., Yuan, Z., Werck-Reichhart, D., Schreiber, L., Wilson, Z.A., and Zhang, D. (2014). ABORTED MICROSPORES acts as a master regulator of pollen wall formation in *Arabidopsis*. *Plant Cell* **26**:1544–1556.
- Yamaguchi, T., Noge, K., and Asano, Y. (2016). Cytochrome P450 CYP71AT96 catalyses the final step of herbivore-induced phenylacetone nitrile biosynthesis in the giant knotweed, *Fallopia sachalinensis*. *Plant Mol. Biol.* **91**:229–239.
- Yang, Y., Li, W., Pang, J., Jiang, L., Qu, X., Pu, X., Zhang, G., and Luo, Y. (2019). Bifunctional cytochrome P450 enzymes involved in camptothecin biosynthesis. *ACS Chem. Biol.* **14**:1091–1096.
- Yoneyama, K., Mori, N., Sato, T., Yoda, A., Xie, X., Okamoto, M., Iwanaga, M., Ohnishi, T., Nishiwaki, H., Asami, T., et al. (2018). Conversion of carlactone to carlactonoic acid is a conserved function of MAX1 homologs in strigolactone biosynthesis. *New Phytol.* **218**:1522–1533.
- Zabel, S., Brandt, W., Porzel, A., Athmer, B., Bennewitz, S., Schäfer, P., Kortbeek, R., Bleeker, P., and Tissier, A. (2021). A single cytochrome P450 oxidase from *Solanum habrochaites* sequentially oxidizes 7-epi-zingiberene to derivatives toxic to whiteflies and various microorganisms. *Plant J.* **105**:1309–1325.
- Zhan, C., Lei, L., Liu, Z., Zhou, S., Yang, C., Zhu, X., Guo, H., Zhang, F., Peng, M., Zhang, M., et al. (2020). Selection of a subspecies-specific diterpene gene cluster implicated in rice disease resistance. *Nat. Plants* **6**:1447–1454.
- Zhang, B., Lewis, K.M., Abril, A., Davydov, D.R., Vermerris, W., Sattler, S.E., and Kang, C. (2020). Structure and function of the cytochrome P450 monooxygenase cinnamate 4-hydroxylase from *Sorghum bicolor*. *Plant Physiol.* **183**:957–973.
- Zhang, X., and Li, S. (2017). Expansion of chemical space for natural products by uncommon P450 reactions. *Nat. Prod. Rep.* **34**:1061–1089.
- Zhang, Y., Cheng, X., Wang, Y., Diez-Simon, C., Flokova, K., Bimbo, A., Bouwmeester, H.J., and Ruyter-Spira, C. (2018). The tomato MAX1 homolog, SIMAX1, is involved in the biosynthesis of tomato strigolactones from carlactone. *New Phytol.* **219**:297–309.
- Zhang, Y., van Dijk, A.D., Scaffidi, A., Flematti, G.R., Hofmann, M., Charnikhova, T., Verstappen, F., Hepworth, J., van der Krol, S., Leyser, O., et al. (2014). Rice cytochrome P450 MAX1 homologs catalyze distinct steps in strigolactone biosynthesis. *Nat. Chem. Biol.* **10**:1028–1033.
- Zhao, M., Cheng, J., Guo, B., Duan, J., and Che, C.T. (2018a). Momilactone and related diterpenoids as potential agricultural chemicals. *J. Agric. Food Chem.* **66**:7859–7872.
- Zhao, Q., Cui, M.Y., Levsh, O., Yang, D., Liu, J., Li, J., Hill, L., Yang, L., Hu, Y., Weng, J.K., et al. (2018b). Two CYP82D enzymes function as flavone hydroxylases in the biosynthesis of root-specific 4'-deoxyflavones in *Scutellaria baicalensis*. *Mol. Plant* **11**:135–148.
- Zi, J., and Peters, R.J. (2013). Characterization of CYP76AH4 clarifies phenolic diterpenoid biosynthesis in the Lamiaceae. *Org. Biomol. Chem.* **11**:7650–7652.