References

1. Zhu, J.K. (2016) Abiotic stress signaling and responses in plants. *Cell* 167, 313–324
2. Takahashi, Y. *et al.* (2020) MAP3Kinase-dependent SnRK2-kinase activation is required for abscisic acid signal transduction and rapid osmotic stress response. *Nat. Commun.* 11, 12
3. Lin, Z. *et al.* (2020) A RAF-SnRK2 kinase cascade mediates early osmotic stress signaling in higher plants. *Nat. Commun.* 11, 613
4. Soma, F. *et al.* (2020) Plant Raf-like kinases regulate the mRNA population upstream of ABA-unresponsive SnRK2 kinases under drought stress. *Nat. Commun.* 11, 1373
5. Katsuta, S. *et al.* (2020) Arabidopsis Raf-like kinases act as positive regulators of subclass III SnRK2 in osmostress signaling. *Plant J.* 103, 634–644
6. Nguyen, Q.T.C. *et al.* (2019) Arabidopsis Raf-like kinase Raf10 is a regulatory component of core ABA signaling. *Mol. Cells* 42, 646–660
7. Boudsocq, M. *et al.* (2007) Different phosphorylation mechanisms are involved in the activation of sucrose non-fermenting 1 related protein kinases 2 by osmotic stresses and abscisic acid. *Plant Mol. Biol.* 63, 491–503
8. Fujii, H. *et al.* (2011) Arabidopsis decuple mutant reveals the importance of SnRK2 kinases in osmotic stress responses *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 108, 1717–1722
9. Saruhashi, M. *et al.* (2015) Plant Raf-like kinase integrates abscisic acid and hyperosmotic stress signaling upstream of SNF1-related protein kinase2. *Proc. Natl. Acad. Sci. U. S. A.* 112, E6388–E6396
10. Jonak, C. *et al.* (2002) Complexity, cross talk and integration of plant MAP kinase signalling. *Curr. Opin. Plant Biol.* 5, 415–424
11. Gonzalez-Guzman, M. *et al.* (2012) Arabidopsis PYR/PYL/RCAR receptors play a major role in quantitative regulation of stomatal aperture and transcriptional response to abscisic acid. *Plant Cell* 24, 2483–2496
12. Vlad, F. *et al.* (2010) Phospho-site mapping, genetic and in planta activation studies reveal key aspects of the different phosphorylation mechanisms involved in activation of SnRK2s. *Plant J.* 63, 778–790

Spotlight

Tracking the Origin and Evolution of Plant Metabolites

Christophe Duplais,[1] Nicolas Papon,[2,3] and Vincent Courdavault[4,*]

Iridoids are monoterpenes that are produced by various plants as chemical defense molecules. Lichman *et al.* recently described the timeline of molecular events that underpin the re-emergence of iridoid biosynthesis in an independent lineage of aromatic plants (catnip). This study represents a benchmark for studying enzyme and metabolite evolution in different clades across the tree of life.

Plant Metabolites

Understanding the origin and evolution of plant metabolites is fundamental to explaining the distribution of natural products among plant families. These metabolites are produced by plants as a response to abiotic and biotic stress, and also mediate inter- and intra-species communication. They are widely used by humans in medicine and agriculture. Mapping the presence and absence of plant metabolites across angiosperms has been undertaken for many years [1], but understanding the molecular mechanisms and evolution of these metabolites remains more challenging. To determine whether a specific chemical family evolved independently in different lineages or arose from the same ancestral pathway, the identification of genes and proteins involved in the biosynthesis of these natural products is an essential prerequisite. For example, in the biosynthesis of plant tropane alkaloids, the enzymes responsible for the tropinone-reduction step are thought to have risen independently across angiosperm species [2]. Conversely, the norcoclaurine synthase for benzylisoquinoline alkaloid biosynthesis in angiosperms is suggested to be the result of monophyletic evolution before the emergence of eudicots [3]. Deciphering the evolution of terpene synthase (TS) enzymes across plant lineages is challenging because terpenes are present in the earliest lineages of land plants that colonized terrestrial habitats [480–430 million years ago (Mya)] [4]. The extensive superfamily of TS enzymes generate high terpenoid chemical diversity that has played a major role in plant diversification and adaptation. In this respect, an impressive new study led by Lichman, Buell, and O'Connor has shed new light on the evolution of a prominent class of monoterpenes, namely iridoids, in a well-known aromatic plant family (the Lamiaceae) [5]. This investigation is a tour de force because it reveals the molecular mechanisms underlying the loss and re-emergence of iridoid biosynthesis in the *Nepeta* lineage.

The Evolution of Iridoids

Iridoids are produced by several plant families and act as a chemical defense against herbivores and plant pathogens. In the Lamiaceae (~200 genera, 7000 species), which comprise seven major subfamilies, iridoids are widely distributed across all subfamilies but are absent from a single subfamily, the Nepetoideae (Figure 1A). Importantly, because iridoids are also present in the sister family of the Lamiaceae, the Verbenaceae, it is likely that genes involved in iridoid biosynthesis evolved from a common ancestor, but the ability to produce iridoids has been lost in the clade of Nepetoideae. However, there is a noteworthy exception because iridoids are found in the Nepetoideae genus *Nepeta*. These plants are known as catmint or catnip owing to the euphoria-inducing effect of the nepetalactone iridoids on the behavior of felines. The intriguing presence of iridoids in Nepetoideae thus raises the question of whether the same enzymes or novel enzymes are involved in the re-emergence of iridoid biosynthesis (Figure 1A).

The structural core of iridoids is a *cis*- or *trans*-fused cyclopentanopyran. Unlike cyclic terpenoids that are generally produced by cyclization of a linear terpene carbocation by TS enzymes, the key step in iridoid biosynthesis is reductive cyclization of 8-oxogeranial by iridoid synthase (ISY) followed by either a Diels–Adler reaction or a Michael addition [6]. ISY was originally discovered in the Madagascar periwinkle (*Catharanthus roseus*, Apocynaceae), a major source of anticancer drugs derived from iridoid monoterpene indole alkaloids [6,7].

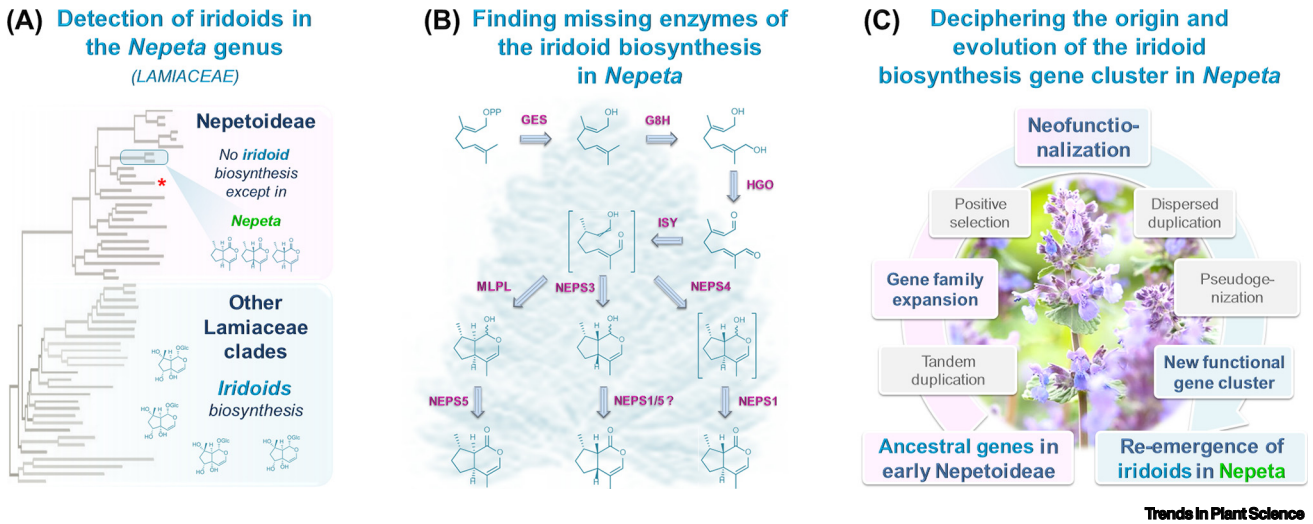

Figure 1. Investigating the Molecular Basis of the Re-Emergence of Iridoid Biosynthesis in the *Nepeta* Lineage. (A) Detection of iridoid metabolites in *Nepeta*. Iridoids are widely distributed in Lamioideae but are absent from the single subfamily Nepetoideae. However, there is a noteworthy exception because iridoids are found in Nepetoideae in the genus *Nepeta*. The phylogenetic tree represents current understanding of the relationships of the Lamiaceae lineages. The red asterisk corresponds to the iridoid nonproducer *Hyssopus officinalis*. (B) The iridoid biosynthetic pathway in *Nepeta*. Nepetalactone biosynthesis in *Nepeta* was reported in [5,12]. This pathway involves geraniol synthase (GES), geraniol 8-hydroxylase (G8H), 8-hydroxygeraniol oxidoreductase (HGO), and iridoid synthase (ISY). Finally, nepetalactol-related short-chain dehydrogenase enzymes (NEPS) or major latex protein-like enzyme (MLPL) cyclizes the reactive intermediate and controls the stereoselectivity of the reaction products. (C) Proposed chronology of events leading to the re-emergence of nepetalactone biosynthesis in *Nepeta*.

Interestingly, the sequence and crystal structure of Madagascar periwinkle ISY reveal that ISY is not similar to the TS family and is instead more closely related to the PRISE (progesterone 5β-reductase/ISY) enzyme family of short-chain NADPH-dependent dehydrogenases [8,9].

To understand the evolution of ISY in aromatic plants, a novel chemical/genomic/phylogenetic approach studying the transcriptomes of 48 Lamiaceae species was recently published [10]. Unexpectedly, functional validation of these ISY candidates revealed that, although ISY activates 8-oxogeranial to generate an enolic intermediate, it does not catalyze the subsequent cyclization into nepetalactone [11]. Instead, a newly identified class of nepetalactol-related short-chain dehydrogenase enzymes (NEPS) mediates cyclization of the reactive intermediate and controls the stereoselectivity of the reaction products [12]. Furthermore, biosynthetic gene clusters composed of *ISY* and *NEPS* were identified by mining *Nepeta* genomes [5]. Interestingly, these clusters also include major latex protein-like genes (*MLPL*) whose encoded proteins act in a similar manner to NEPS to generate a *cis-trans* nepetalactone stereoisomer (Figure 1B).

With these key pieces of information in hand, Lichman *et al.* investigated the loss and re-emergence of iridoid biosynthesis during the evolution of the Nepetoideae. First, genome sequences of *Nepeta cataria* and *Nepeta mussinii* were compared with those of the iridoid nonproducer *Hyssopus officinalis* (indicated by a red asterisk in Figure 1A); this confirmed that the absence of iridoids results from loss of the *ISY* gene in this latter species, as well as in other Nepetoideae for which omic data are available. Surprisingly, it also directly correlates nepelactone biosynthesis with regain of ISY in *N. cataria* and *N. mussinii*. Furthermore, phylogenetic analyses clearly indicate that the *ISY* genes of *Nepeta* form a distinct clade in the Lamioideae subfamily, which strongly suggests distinct and parallel evolution of ISYs. Second, the evolution of *ISY* in *Nepeta* was assessed using ancestral sequence reconstruction to infer the PRISE phylogeny of the enzyme. This comparative phylogenetic method was then combined with positive selection analysis and screening of *in vitro* activities of extant and predicted ancestral PRISE enzymes. Overall, the key findings on the re-emergence of ISY argue that an ancestral enzyme with minor ISY side-activity gradually evolved into a novel iridoid biosynthetic enzyme with high ISY activity following gene duplication and selection (Figure 1C).

The tour de force was to compare the evolution and diversification of NEPS with the emergence of ISY activity and to elucidate the chronology of the events leading to assembly of the nepetalactone gene cluster. By comparing PRISE and NEPS chronograms, the authors deduced that the gain of the most recent common ancestor of the NEPS gene was concomitant to the second gene duplication of the ISY ancestor (~25 Mya), whereas the NEPS family expansion occurred at the same time that ISY relative

CellPress
REVIEWS

activity dramatically increased and P5βR activity was lost (20–9 Mya). Ultimately, a dispersed duplication event allowed ISY to integrate into the *NEPS* locus, and the original copy at the *PRISE* locus turned into pseudogenized since it became redundant (9 Mya to today). These discoveries strongly suggest that ISY and NEPS catalytic activities coevolved, with strong interplay between corresponding genomic regions (Figure 1C).

The evolution of iridoids in Lamiaceae thus stands out as a fascinating example of tracing the origins of plant metabolites and the re-emergence of their biosynthesis in *Nepeta*. The present study provides unprecedented insights suggesting that this phenomenon relies on repeated and innovative evolution, thereby further widening our knowledge of the production of nepetalactones versus iridoids in the wider mint family. The proposed chronology of enzyme selection and diversification also suggests that the formation of gene clusters may not drive metabolic innovation, but instead organizes subsequent enzyme evolution under strong initial pressure. Now highlighted, this complex evolutionary story raises puzzling new questions ranging from the role of protein–protein interactions between ISY and NEPS/MLPL during the stereoselective formation of nepetalactones, to the biotic and abiotic factors that are responsible for the co-evolution of ISY and NEPS. In addition, because iridoids also occur across different insect taxa, comparison of plant and insect iridoid synthases will provide a crucial entry point to address the evolutionary convergence of the production of these common compounds. The evolution of iridoids represents a remarkable model for studying enzyme and metabolite evolution across the tree of life.

Acknowledgments

We thank Corrie Moreau for useful comments on the manuscript. We acknowledge funding from the EU Horizon 2020 research and innovation program (MIAMi project-Grant agreement N°814645), ARD2020 Biopharmaceutical program of the Région Centre Val de Loire (BioPROPHARM, CatharSIS, and ETOPOCentre projects), le "Pays de la Loire" CRHoMic project, La Ligue Contre le Cancer (Yeast4LiFE), and le Studium (Consortium fellowship). We are also grateful for the financial support of an 'Investissement d'Avenir' grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01).

[1]Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 8172 Ecologie des Forêts de Guyane (EcoFoG), AgroParisTech, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Institut National de la Recherche Agronomique (INRA), Université des Antilles and Université de Guyane, Cayenne, France
[2]Host–Pathogen Interaction Study Group (GEIHP, Équipe d'Accueil EA 3142), Université d'Angers and Université de Brest, Angers, France
[3]Cellular Interactions and Therapeutic Applications (ICAT), Structure Féderative de Recherche (SFR) 4208, Université d'Angers, Angers, France
[4]Biomolécules et Biotechnologies Végétales (BBV) EA 2106, Université de Tours, Tours, France

*Correspondence:
vincent.courdavault@univ-tours.fr (V. Courdavault).

https://doi.org/10.1016/j.tplants.2020.08.010

References

1. Wink, M. (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* 64, 3–19
2. Jirschitzka, J. *et al.* (2012) Plant tropane alkaloid biosynthesis evolved independently in the Solanaceae and Erythroxylaceae. *Proc. Natl. Acad. Sci. U. S. A.* 109, 10304–10309
3. Liscombe, D.K. *et al.* (2005) Evidence for the monophyletic evolution of benzylisoquinoline alkaloid biosynthesis in angiosperms. *Phytochemistry* 64, 1374–1393
4. Lange, B.M. (2015) The evolution of plant secretory structures and emergence of terpenoid chemical diversity. *Annu. Rev. Plant Biol.* 66, 139–159
5. Lichman, B.R. *et al.* (2020) The evolutionary origins of the cat attractant nepetalactone in catnip. *Sci. Adv.* 6, eaba0721
6. Geu-Flores, F. *et al.* (2012) An alternative route to cyclic terpenes by reductive cyclization in iridoids synthesis. *Nature* 492, 138–142
7. Kries, H. *et al.* (2017) Inverted stereocontrol of iridoid synthase in snapdragon. *J. Biol. Chem.* 292, 14659–14667
8. Kries, H. *et al.* (2012) Structural determinants of the reductive terpene cyclization in iridoid biosynthesis. *Nat. Chem. Biol.* 12, 6–8
9. Nguyen, T.-D. and O'Connor, S.E. (2020) The progesterone 5β-reductase/Iridoid synthase family: a catalytic reservoir for specialized metabolism across land plants. *ACS Chem. Biol.* Published online June 5, 2020. https://doi.org/10.1021/acschembio.0c00220
10. Mint Evolutionary Genomics Consortium (2018) Phylogenomic mining of the mints reveals multiple mechanisms contributing to the evolution of chemical diversity in Lamiaceae. *Mol. Plant* 11, 1084–1096
11. Sherden, N.H. *et al.* (2018) Identification of iridoid synthases from *Nepeta* species: iridoid cyclization does not determine nepetalactone stereochemistry. *Phytochemistry* 145, 48–56
12. Lichman, B.R. *et al.* (2019) Uncoupled activation and cyclization in catmint reductive terpenoid biosynthesis. *Nat. Chem. Biol.* 15, 71–79

Spotlight

Does Karrikin Signaling Shape the Rhizomicrobiome via the Strigolactone Biosynthetic Pathway?

Fahad Nasir,[1] Weiqiang Li,[2] Lam-Son Phan Tran,[3,4,*] and Chunjie Tian[1,5,*]

A recent study by Choi *et al.* provides evidence of the interaction between the karrikin (KAR) signaling and strigolactone (SL) biosynthetic pathways. Since SLs shape rhizomicrobiome composition, it is of interest to determine whether KAR signaling could affect rhizomicrobiome composition by improving the synthesis of root-derived SLs to support climate-smart agriculture.

Components of SL and KAR Signaling Pathways

SLs, the newest class of phytohormones, were first characterized as rhizosphere signaling molecules due to their ability to promote seed germination in parasitic plants [1]. Subsequently, SLs were demonstrated to play a role in the interaction of host plants with beneficial arbuscular mycorrhizal (AM) fungi and nitrogen-fixing rhizobia [1] and in the abiotic and biotic stress responses of various plant species [1]. In rice (*Oryza sativa*), SLs are perceived by an α/β-hydrolase receptor named OsDWARF 14 (OsD14), which is a homolog of arabidopsis (*Arabidopsis thaliana*) AtD14