PLANT SCIENCE

Plant Gene Clusters and Opiates

A variety of poppy that produces high amounts of an alkaloid opiate requires a cluster of genes that encode key biosynthetic enzymes.

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Plant natural products have a profound impact on human health and are prime targets for drug development, but full realization of their clinical potential is often curtailed because these compounds are typically produced in small amounts in slow-growing plant species. Improving production levels is essential for bringing new plant compounds into the drug development pipeline, but such improvements demand an understanding of the fundamental biological processes underlying complex natural product synthesis in plants. On page 1704 of this issue, Winzer et al. (1) define genes encoding the biosynthetic pathway for noscapine, a medicinal alkaloid produced by opium poppy.

Plants synthesize hundreds of thousands of diverse natural products that play key roles in their development and responses to environmental stress. Many of these metabolites also exhibit potent bioactivity or toxicity against insects and herbivores and provide plants with a selective advantage in these interactions. Although substantial metabolic cost is associated with the production of specialized plant metabolites, their biosynthetic pathways are strongly favored and maintained by natural selection. This continuous selection process has generated plant compounds with a range of pharmaceutical uses. Indeed, two-thirds of compounds now in clinical use originated from discoveries related to specialized plant metabolites (2). However, with few exceptions, a specific medicinally important compound is produced by only a handful of the estimated 400,000 plant species. Moreover, most medicinal species are poorly studied and are largely intractable to standard biochemical and genetic approaches. Recent advances in high-throughput sequencing, metabolomics, bioinformatics, and viral-based plant gene silencing have provided new avenues for rapidly advancing our understanding of biosynthetic pathways for such taxonomically restricted plant natural products (3).

Noscapine is a nonaddictive alkaloid that has been safely used for decades as an orally administered antitussive and has recently

Plant species	Compound produced	Compound class	Likely function in host plant
Lotus japonicus	Linamarin	Cyanogenic glycoside	Herbivore defense
Manihot esculenta	Linamarin	Cyanogenic glycoside	Herbivore defense
Sorghum bicolor	Dhurrin	Cyanogenic glycoside	Herbivore defense
Zea mays	DIMBOA	Cyclic hydroxamic acid	Insect and microbial defense
Avena sativa	Avenacin A-1	Triterpene saponin	Antifungal
Oryza sativa	Phytocassane A	Diterpene phytoalexin	Antifungal
Oryza sativa	Momilactone A	Diterpene phytoalexin	Antifungal
Arabidopsis thaliana	Thalianol	Triterpene	Unknown
Arabidopsis thaliana	Marneral	Triterpene	Unknown
Papaver somniferum	Noscapine	Alkaloid	Unknown

Specialized metabolites. The biosynthetic pathways of the metabolites listed are partially or fully encoded in clusters in the genomes of the indicated plant species (1, 5–11).

shown promising anticancer activity as a tubulin polymerization inhibitor and inducer of apoptosis (4). As an alkaloid, it is one of a group of more than 12,000 specialized plant metabolites present in about 20% of plant species and characterized by the presence of a nitrogen-containing heterocyclic ring. There is enormous chemical diversity among the alkaloids, with more than 20 biochemically and structurally distinct classes recognized. This chemical complexity makes characterizing the biosynthesis of individual alkaloids particularly difficult.

The elucidation of the noscapine pathway reported by Winzer et al. was greatly accelerated by their discovery that the biosynthetic genes are physically clustered in the poppy genome. By crossing poppy varieties containing high levels of morphine (a highly addictive alkaloid in the opium poppy) or noscapine, the authors show that the biochemically complex noscapine trait segregates as a single Mendelian locus. By comparing the transcriptomes (all of the messenger RNA molecules produced), metabolite profiles, and genomes of varieties that produce high morphine, high thebaine (another opiate alkaloid), and high noscapine, they identified 10 genes that are highly expressed in tissues of the high-noscapine variety but absent from the tissues and genomes of the high-morphine and high-

thebaine varieties. By contrast, known genes for morphine synthesis were present and similarly expressed in all three varieties. A 401-kb genomic interval specific to the noscapine variety was found to contain all 10 genes, six of which were shown—by virus-induced gene silencing followed by metabolomics analysis of poppy, or by gene expression and biochemical assays in yeast—to encode specific steps of the noscapine pathway. Although some members of the noscapine biosynthetic cluster appear to have arisen by recent gene duplication, the majority of genes encode unrelated proteins that catalyze distinct reactions in the pathway (e.g., divergent cytochrome P450s and methyltransferases). The noscapine biosynthetic cluster adds to a growing body of knowledge about the involvement of gene clusters in the synthesis of specialized plant metabolites (see the figure).

Since the first identification of a tightly linked genetic cluster for the biosynthesis of cyclic hydroxamic acid in maize in 1997 (5), 10 additional instances of gene clusters for specialized metabolism in plants have been reported from seven other plant species (1, 6–11). Specialized plant metabolism gene clusters occur relatively infrequently on a genome-wide basis, but as more are characterized, they will become easier to identify. Although we still do not understand the

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Published by AAAS

forces that generate and maintain gene clusters for specialized plant metabolism (*12*), such clusters, once identified, are invaluable for decoding of complex, taxonomically restricted pathways in plants. One need only compare the pace of dissecting noscapine synthesis to the nearly two decades required to fully decipher the pathway for morphine to fully appreciate the power of such metabolic Rosetta stones. Low-cost, high-throughput sequencing has increasingly driven biosynthetic gene cluster identification in plants;

as our ability to apply molecular genetic tools (such as virus-induced gene silencing) expands to more nonmodel plant species with unique biochemistries, we can anticipate that even more dark recesses of specialized plant metabolism will be illuminated.

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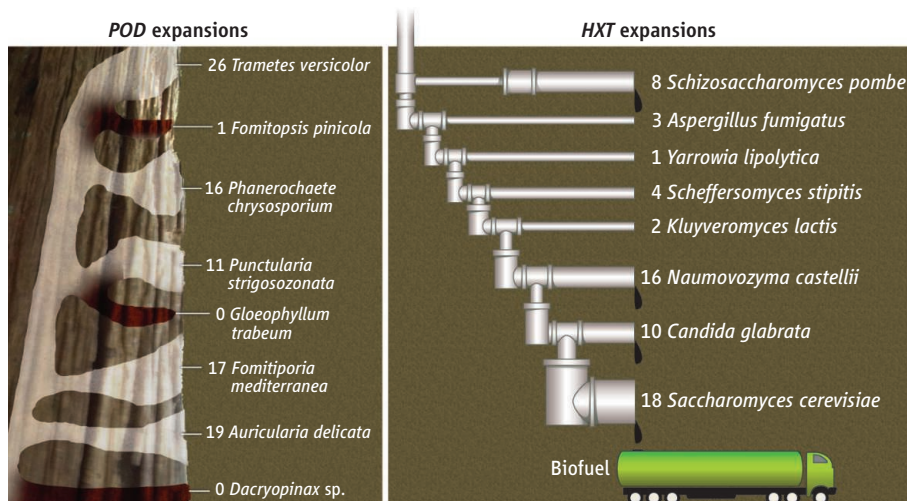
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EVOLUTION

Endless Rots Most Beautiful

Chris Todd Hittinger

Deep phylogenetic sampling of 31 fungal genomes, including 12 new ones, reveals how wood-decaying enzymes evolved in white rot.

Fungal rots that decay wood were not prominent among the "endless forms most beautiful" that Darwin chronicled, but if he had known of the biochemical and evolutionary processes at work, they might have been. Woody plants fix an extraordinary amount of carbon during their lifetimes, building towering trees of decay-resistant lignocellulose. On page 1715 of this issue, Floudas *et al.* performed deep phylogenetic sampling of fungal genomes to describe how white rot Agaricomycetes fungi have evolved an arsenal of enzymes to degrade lignin and unlock its stored carbon (*1*).

As plants invaded land, lignin provided the rigidity necessary for vascular plants to grow above their rivals and move water and nutrients over long distances (*2*). Lignin is a dizzying web of polymerized phenylalanine derivatives with dozens of combinations of modifications and cross-links that make wood structurally sound and render it inaccessible to all but the most persistent chemical and biological assaults (*2–5*). The success of lignin-rich plants in the swamp forests of the Carboniferous Period created many of the coal-rich deposits that fueled the Industrial Revolution. But little carbon is buried today, in part because of white rot fungi. Floudas *et al.*'s analysis of 12 newly sequenced species of Agaricomycotina provides a treasure trove of wood-decaying enzymes to test and industrialize (*5*, *6*) as well as remarkable insights into the genomics of adaptive shifts, gene duplication and diversification, and parallel evolution.

Parallel gene family expansions in fungi. (**Left**) Approximate evolutionary relationships (left to right, 300 million years ago to the present) and numbers of *POD* genes (also represented by width) in white and brown rot basidiomycetes. (**Right**) Relationships and numbers of *HXT* genes in ascomycetes; ethanol drops indicate taxa that ferment aerobically, including *S. cerevisiae*, the main producer of liquid biofuels.

POD expansions
- 26 *Trametes versicolor*
- 1 *Fomitopsis pinicola*
- 16 *Phanerochaete chrysosporium*
- 11 *Punctularia strigosozonata*
- 0 *Gloeophyllum trabeum*
- 17 *Fomitiporia mediterranea*
- 19 *Auricularia delicata*
- 0 *Dacryopinax* sp.

HXT expansions
- 8 *Schizosaccharomyces pombe*
- 3 *Aspergillus fumigatus*
- 1 *Yarrowia lipolytica*
- 4 *Scheffersomyces stipitis*
- 2 *Kluyveromyces lactis*
- 16 *Naumovozyma castellii*
- 10 *Candida glabrata*
- 18 *Saccharomyces cerevisiae*

Biofuel

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Broad taxonomic and ecological sampling of rots allowed Floudas *et al.* to use state-of-the-art phylogenomic and ancestral state inference to trace the history of the Agaricomycetes and their myriad lignocellulolytic enzymes. Fifteen gene families deviate from the null expectation of a random birth-death process of gene duplication and loss, and instead exhibit significant lineage-specific expansions and contractions. For example, glycoside hydrolases, multicopper oxidases, and dye-decolorizing peroxidases expanded during the evolution of the lignin-degrading lifestyle of white rots, while contractions occurred in brown rot lineages that do not appreciably degrade lignin.

The most striking expansions and contractions are apparent in the fungal class II

peroxidases (PODs), which are the primary lignin-degrading enzymes in white rots (*7*). The common ancestor of Agaricomycetes is inferred to have been a white rot with a modest repertoire of manganese peroxidase PODs. Indeed, nearly all modern white rot fungi possess several manganese peroxidases, whereas only a few species possess lignin peroxidases and versatile peroxidases capable of directly oxidizing aromatic rings. The branching pattern of the Agaricomycetes suggests that PODs independently expanded along multiple white rot lineages through gene duplication and independently contracted to zero or one *POD* genes in at least three brown rot lineages (see the figure). The few PODs found in non–white rot fungi all lack key sequences associated with lignolytic

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