May 20, 2022

Plant Biology

Figuring out the evolved chemistry of fig trees

by **Kaouthar Eljounaidi**[1] | Postdoctoral Research Fellow

[1]: University of York, United Kingdom

This Break was edited by Quentin Laurent, *Senior Scientific Editor* - TheScienceBreaker

ABSTRACT

Fig trees (Ficus carica) produce furanocoumarins, a class of small organic molecules with various medicinal and agricultural applications. Villard et al. studied the enzyme catalysing the first synthetic step in the production of these molecules. They revealed how this enzyme emerged recently and independently within the Ficus lineage in a mechanism called convergent evolution.

Image credits: ryckyy21 -- Pixabay

Furanocoumarins are small organic molecules produced by plants and known to play defense roles against pathogens and herbivores. Interestingly, some of them are also potential treatments for cancer or vitiligo. These compounds are found in specific but surprisingly distantly related plant families, such as in the parsley family (e.g. bishop's weed) and mulberry family (e.g. common fig).

To produce these molecules, plants use some enzymes that act as biological catalysts, molecular factories that construct these chemicals from simple precursors. Villard and colleagues investigated the genes that code for these enzymes to understand how the synthesis of furanocoumarins emerged independently in distant plant lineages.

The authors examined the first chemical step in the synthesis of linear furanocoumarins in common fig (*Ficus carica*). They already had two clues to examine this system: first, in bishop's weed plant, the equivalent enzyme catalysing the same reaction belongs to the cytochrome P450 enzyme superfamily, enzymes known for their versatility and for contributing

immensely to the chemical diversity in plants. Then, in common fig, furanocoumarins are more concentrated in the leaf's stalks compared to the trunk and fruits. This means that the concentrations of the target enzyme will likely be higher in the leaf's stalks. This directed the search toward enzymes which belong to the cytochrome P450 family and that would be present in the leaf's stalks.

The starting point was looking into the common fig RNA library. RNAs are intermediate gene products that are the first molecules produced in the making of a protein. Through their search, they found nine candidate genes that match the desired criteria. Next, the function of the candidate cytochrome P450 genes was tested in the lab through enzymatic assays. The authors discovered that one of the candidates (CYP76F112) could perform the first step of furanocoumarin synthesis. Further in-depth characterisation of the enzyme's activity showed that this conversion occurred with high affinity and efficiency. This indicates adaptation to the presence of the substrate in the plant at low concentrations, which requires highly efficient enzymes.

Subsequently, the authors studied the enzyme mechanisms using a method called site-directed-mutagenesis. It consists of targeted changes to the enzyme coding sequence, to study the importance of the modified region. When investigating the enzyme activities after these changes, the authors discovered that an amino acid, named M117, seemed to be critical for the activity of the enzyme. This amino acid is located next to the substrate binding site and assumed to shape the binding region, thus playing a key role in the substrate specificity and selectivity.

Next, the authors investigated how the newly discovered enzyme CYP76F112 emerged in fig tree lineage by building phylogenetic trees. These are evolutionary trees built based on the comparison of a set of homologous DNA or protein sequences in different organisms. Their pattern of branching provides clues on how specific genes evolved from a series of common ancestors. The authors built an evolutionary tree of homologs to CYP76F112 gene from species belonging to different plant families, such as mulberries and hemps. The tree showed that CYP76F112 is placed within a group of branches that includes exclusively *Ficus* sequences. This finding supports the hypothesis that CYP76F112 emerged recently and independently within the *Ficus* lineage. This mechanism of independent emergence of the same feature in distant branches is called convergent evolution.

In summary, the authors have identified a new cytochrome P450 enzyme that catalyses the first step of furanocoumarin synthesis. Using interdisciplinary approaches such as evolutionary trees and enzyme characterisation studies, they elucidated how this enzyme evolved and highlighted important parts for its activity. The discovered enzyme is highly stable and efficient, thus could be used as a versatile biocatalyst for sustainable synthetic chemistry applications.

Furanocoumarins are considered promising drug candidates against diseases such as cancer and vitiligo. They also hold promises as sustainable biocontrol tools to reduce the use of synthetic pesticides. Uncovering the synthetic steps of these molecules will help increasing their availability and accessibility, for various medicinal and agrochemical applications.