

Phylogenetic patterns in leaf wax *n*-alkane hydrogen isotope composition can be observed in spatially separated parts of the biosynthetic pathway

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Background

The hydrogen stable isotope composition ($\delta^2\text{H}$) of plant derived leaf wax *n*-alkanes ($\delta^2\text{H}_{\text{alkane}}$) is used to reconstruct past climate, as $\delta^2\text{H}_{\text{alkane}}$ reflects environmentally driven source- and leaf-water $\delta^2\text{H}$ values. However, $\delta^2\text{H}_{\text{alkane}}$ values can strongly vary among species at a single geographic location suggesting strong variation in species-specific biosynthetic ^2H -fractionation. Thus, species-specific variation in $\delta^2\text{H}_{\text{alkane}}$ values may cloud the interpretation of $\delta^2\text{H}_{\text{alkane}}$ in climate reconstructions.

Objectives

- 1) Determine variation in $\delta^2\text{H}_{\text{alkane}}$ values across species grown in a single location, and test if this is related to phylogeny (**Fig. 1**).
- 2) Distinguish sources of variation in species-specific $\delta^2\text{H}_{\text{alkane}}$ values between chloroplast and cytosol along the biosynthetic pathway (**Fig. 2**).

Conclusions

- 1) Large species-specific variation in $\delta^2\text{H}_{\text{alkane}}$ values needs to be accounted for in palaeoclimatological reconstructions.
 - 2) Species-specific $\delta^2\text{H}_{\text{alkane}}$ values are shaped in both chloroplast and cytosol, but the extent of this depends on phylogeny.
- ❖ Potential new tool for understanding evolution of plant lipid metabolism?

Results & Discussion

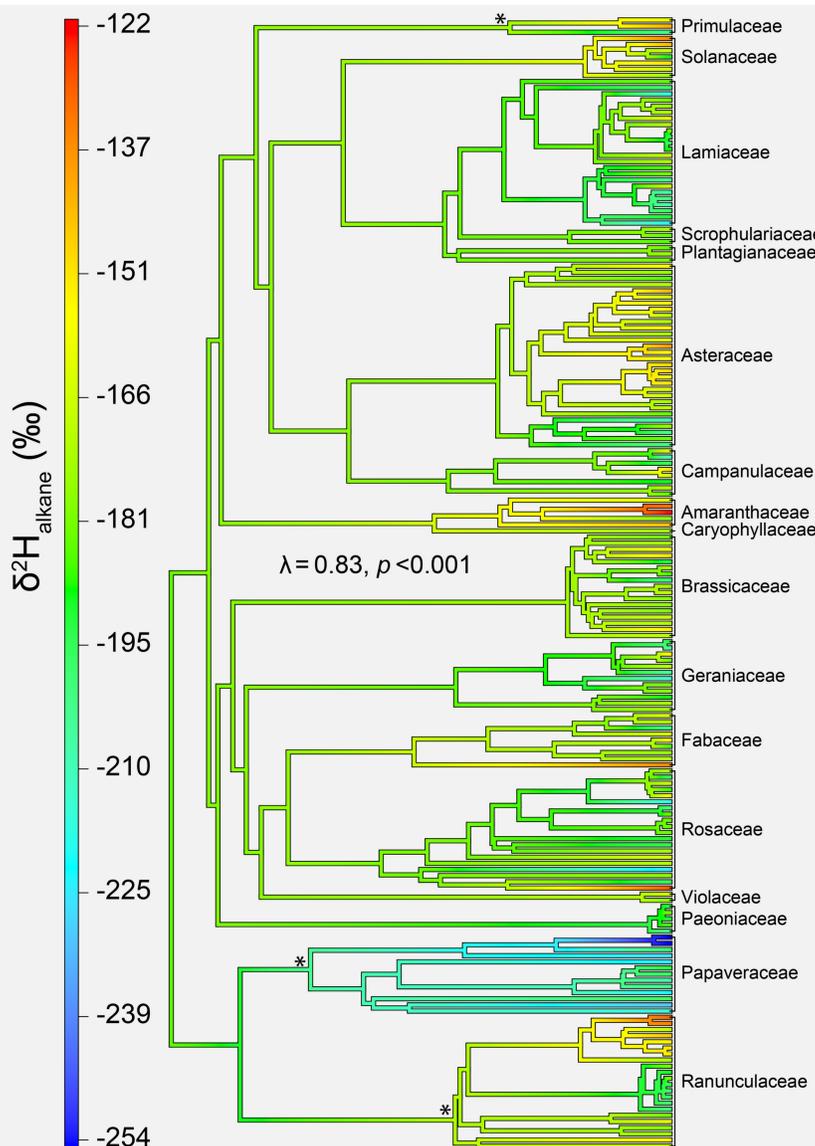


Figure 1: $\delta^2\text{H}_{\text{alkane}}$ values mapped as color gradient along branches of eudicot phylogeny. *Branches where species were sampled more densely in 2020.

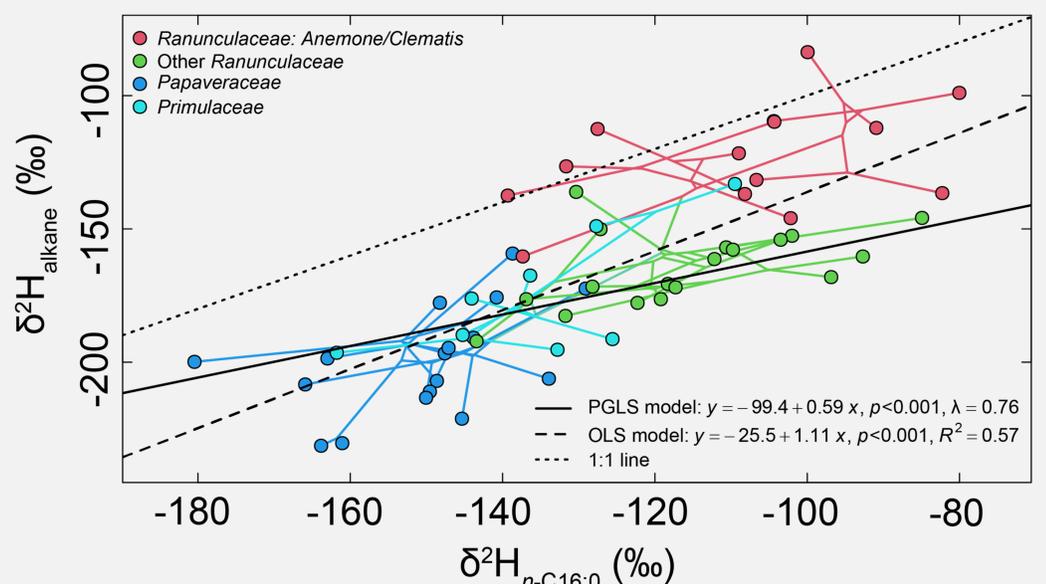


Figure 2: $\delta^2\text{H}_{\text{alkane}}$ values plotted against chloroplast produced precursor $\delta^2\text{H}_{n\text{-C}16:0}$ values. Each point is a single species and lines between points are branches of the phylogenetic tree. Abbreviations: PGLS, phylogenetic generalized least squares; OLS, ordinary least squares.

- 1) $\delta^2\text{H}_{\text{alkane}}$ values significantly evolved along the phylogeny and can vary up to 130 ‰ across eudicot species (**Fig. 1**), while leaf water $\delta^2\text{H}$ values varied only up to 35 ‰ across species (data not shown).
- 2) Chloroplast produced precursor, $\delta^2\text{H}_{n\text{-C}16:0}$ values can explain a large part of the variation in $\delta^2\text{H}_{\text{alkane}}$, but additional variation is introduced in the cytosolic part of the biosynthetic pathway, which is dependent on phylogeny (**Fig. 2**).

Methods

All plants were sampled from the University of Basel botanical garden.

- 1) $\delta^2\text{H}_{\text{alkane}}$ values measured in leaves of 184 eudicot plant species in 2019.
- 2) $\delta^2\text{H}_{\text{alkane}}$ values and $\delta^2\text{H}$ values of chloroplast produced precursor (palmitic acid; $\delta^2\text{H}_{n\text{-C}16:0}$) measured in leaves of 58 species in 2020.

Acknowledgements:

Svenja Förster for support with compound extractions and University of Basel botanical garden staff for help with sampling

Funding:

ERC consolidator grant 724750 HYDROCARB to A. Kahmen

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