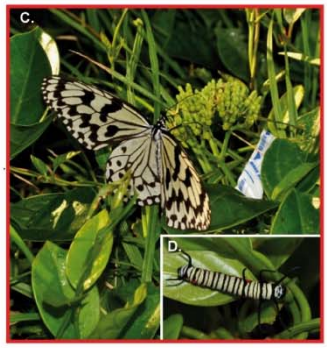

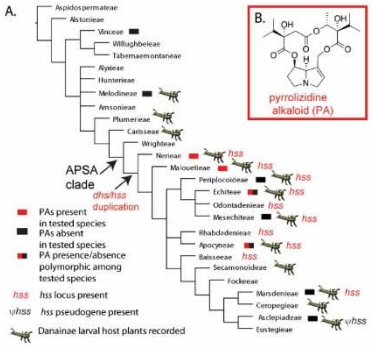

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Biochemische Ökologie & Molekulare Evolution

September, 2018 Master's thesis AG Ober

Exploring the catalytic landscape that separates two paralogous enzymes

The two paralogous enzymes, deoxyhypusine synthase (DHS) and homospermidine synthase (HSS), functionally clearly diverged since their origination. While the DHS is involved in primary metabolism and essential for all eukaryotes, the HSS catalyzes a key step in pyrrolizidine alkaloid (PA) biosynthesis and is only present is specific land plant families. PAs are important plant defense compounds against herbivores like milkweed butterflies and part of the so-called secondary or specialized metabolism.

The difference in function of DHS and HSS basically involves a shift in substrate preference. While the DHS uses an eukaryotic initiation factor as substrate, HSS prefers putrescine. Phylogenetic analysis indicates that two amino acid substitutions are mainly involved in this shift of substrate preference. We now want to study the influence of these residues on the catalytic properties of HSS and DHS enzymes by creating mutants of the enzymes and subsequently characterize their catalytic properties.

Techniques which will be applied: site-directed mutagenesis, heterologous protein expression and subsequent purification, activity assays and quantification of reaction products by HPLC-UV.

For more background information please read:

Livshultz, T., Kaltenegger, E., Straub, S., Weitemier, K., Hirsch, E., Koval, K., Mema, L., Liston, A. (2018). Evolution of pyrrolizidine alkaloid biosynthesis in Apocynaceae: revisiting the defence de-escalation hypothesis. New Phytologist. 218: 762-773.

Kaltenegger, E., Eich, E., Ober, D. (2013). Evolution of homospermidine synthase in the Convolvulaceae – a story of gene duplication, gene loss, and periods of various selection pressures. Plant Cell, 25: 1213-1227

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